Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 35. Document ID: US 6057104 A

L1: Entry 35 of 65 File: USPT May 2, 2000

US-PAT-NO: 6057104
DOCUMENT-IDENTIFIER: US 6057104 A

TITLE: Disruption of the mammalian Rad51 protein and disruption of proteins that associate with mammalian Rad51 for hindering cell proliferation

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 36. Document ID: US 6045804 A

L1: Entry 36 of 65 File: USPT Apr 4, 2000

US-PAT-NO: 6045804
DOCUMENT-IDENTIFIER: US 6045804 A

TITLE: Method for detecting B. burgdorferi infection

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 37. Document ID: US 6037125 A

L1: Entry 37 of 65 File: USPT Mar 14, 2000

US-PAT-NO: 6037125
DOCUMENT-IDENTIFIER: US 6037125 A

TITLE: Disruption of the mammalian RAD51 protein and disruption of proteins that associate with mammalian RAD51 for hindering cell proliferation and/or viability of proliferating cells

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 38. Document ID: US 6030954 A

L1: Entry 38 of 65 File: USPT Feb 29, 2000

US-PAT-NO: 6030954
DOCUMENT-IDENTIFIER: US 6030954 A

TITLE: Targeted delivery of poly- or oligonucleotides to cells

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 5. Document ID: US 20020103151 A1

L1: Entry 5 of 65 File: PGPB Aug 1, 2002

PGPUB-DOCUMENT-NUMBER: 20020103151
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020103151 A1

TITLE: Methods and compositions for immunomodulation

PUBLICATION-DATE: August 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gorczynski, Reginald M.	Willowdale		CA	
Clark, David A.	Burlington		CA	

US-CL-CURRENT: 514/44; 514/12

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 6. Document ID: US 20020086840 A1

L1: Entry 6 of 65 File: PGPB Jul 4, 2002

PGPUB-DOCUMENT-NUMBER: 20020086840
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020086840 A1

TITLE: Use of Rad51 inhibitors for p53 gene therapy

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Zarling, David A.	Menlo Park	CA	US	
Reddy, Gurucharan	Fremont	CA	US	

US-CL-CURRENT: 514/44; 424/155.1, 514/12

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 7. Document ID: US 20020086011 A1

L1: Entry 7 of 65 File: PGPB Jul 4, 2002

PGPUB-DOCUMENT-NUMBER: 20020086011
PGPUB-FILING-TYPE: new

NAME	CITY	STATE	COUNTRY	RULE-47
Monia, Brett P.	Encinitas	CA	US	
Cook, Phillip Dan	Fallbrook	CA	US	
Crooke, Stanley T.	Carlsbad	CA	US	
Lima, Walter	San Diego	CA	US	
Wu, Hongjiang	Carlsbad	CA	US	

US-CL-CURRENT: 435/199; 514/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 20. Document ID: US 20010036929 A1

L1: Entry 20 of 65 File: PGPB Nov 1, 2001

PGPUB-DOCUMENT-NUMBER: 20010036929
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010036929 A1

TITLE: Xrcc3 is required for assembly of Rad51-complexes in vivo

PUBLICATION-DATE: November 1, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Weichselbaum, Ralph R.	Chicago	IL	US	
Bishop, Douglas K.	Chicago	IL	US	

US-CL-CURRENT: 514/44; 424/649, 514/34

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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Search Results - Record(s) 1 through 10 of 65 returned.

☐ 1. Document ID: US 20020111590 A1

L1: Entry 1 of 65 File: PGPB Aug 15, 2002

PGPUB-DOCUMENT-NUMBER: 20020111590
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020111590 A1

TITLE: Medical devices, drug coatings and methods for maintaining the drug coatings thereon

PUBLICATION-DATE: August 15, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Davila, Luis A.	Pleasanton	CA	US	
Lentz, David Christian	Weston	FL	US	
Llanos, Gerard H.	Stewartsville	NJ	US	
Mendez, Jorge Orlando	Miami	FL	US	
Narayanan, Pallassana V.	Belle Mead	NJ	US	
Pelton, Alan Roy	Fremont	CA	US	
Roller, Mark B.	North Brunswick	NJ	US	
Scheidt, Karl K.	Pembroke Pines	FL	US	
Scopelianos, Angelo George	Whitehouse Station	NJ	US	
Shaw, William Douglas JR.	Miami	FL	US	
Silver, James H.	Redwood City	CA	US	
Spaltro, John	Asbury	NJ	US	
Trepanier, Christine	Fremont	CA	US	
Wilson, David J.	Ft. Lauderdale	FL	US	

US-CL-CURRENT: 604/265; 623/1.15, 623/1.42

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 20020110892 A1

L1: Entry 2 of 65 File: PGPB Aug 15, 2002

PGPUB-DOCUMENT-NUMBER: 20020110892
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020110892 A1

TITLE: Human RNase H and compositions and uses thereof

PUBLICATION-DATE: August 15, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Crooke, Stanley T.	Carlsbad	CA	US	
Lima, Walter F.	San Diego	CA	US	
Wu, Hongjiang	Carlsbad	CA	US	

US-CL-CURRENT: 435/199; 424/94.61, 435/6, 435/69.1, 435/91.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC
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☐ 3. Document ID: US 20020107216 A1

L1: Entry 3 of 65 File: PGPB Aug 8, 2002

PGPUB-DOCUMENT-NUMBER: 20020107216
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020107216 A1

TITLE: Integrin-linked kinase and its use

PUBLICATION-DATE: August 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Dedhar, Shoukat	Richmond		CA	
Hannigan, Greg	Toronto		CA	
Yee, Arthur	Burnaby		CA	

US-CL-CURRENT: 514/44; 435/194, 435/455, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC
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☐ 4. Document ID: US 20020103526 A1

L1: Entry 4 of 65 File: PGPB Aug 1, 2002

PGPUB-DOCUMENT-NUMBER: 20020103526
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020103526 A1

TITLE: Protective coating for stent

PUBLICATION-DATE: August 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Steinke, Tom	San Diego	CA	US	

US-CL-CURRENT: 623/1.11

DOCUMENT-IDENTIFIER: US 20020086011 A1

TITLE: Methods and compositions for modulating autoimmunity

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gorczynski, Reginald	Willowdale		CA	

US-CL-CURRENT: 424/130.1; 424/178.1, 514/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 8. Document ID: US 20020076712 A1

L1: Entry 8 of 65 File: PGPB Jun 20, 2002

PGPUB-DOCUMENT-NUMBER: 20020076712
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020076712 A1

TITLE: Human RNase H and compositions and uses thereof

PUBLICATION-DATE: June 20, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Crooke, Stanley T.	Carlsbad	CA	US	
Lima, Walter	San Diego	CA	US	
Wu, Hongjiang	Carlsbad	CA	US	

US-CL-CURRENT: 435/6; 435/199, 435/320.1, 435/325, 435/69.1, 514/44, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 9. Document ID: US 20020068711 A1

L1: Entry 9 of 65 File: PGPB Jun 6, 2002

PGPUB-DOCUMENT-NUMBER: 20020068711
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020068711 A1

TITLE: Arrest of proliferation of highly glycolytic tumors

PUBLICATION-DATE: June 6, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Pedersen, Peter L.	Columbia	MD	US	
Mathupala, Saroj P.	Clinton Twp	MI	US	

US-CL-CURRENT: 514/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 10. Document ID: US 20020064554 A1

L1: Entry 10 of 65 File: PGPB May 30, 2002

PGPUB-DOCUMENT-NUMBER: 20020064554
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020064554 A1

TITLE: Radiation sensitive liposomes

PUBLICATION-DATE: May 30, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
O'Brien, David F.	Tucson	AZ	US	
McGovern, Kathy A.	Tucson	AZ	US	
Bondurant, Bruce	Tucson	AZ	US	
Sutherland, Robert	Menlo Park	CA	US	

US-CL-CURRENT: 424/450

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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Term	Documents
RAD.DWPI,EPAB,JPAB,USPT,PGPB.	21036
RADS.DWPI,EPAB,JPAB,USPT,PGPB.	2562
"51".DWPI,EPAB,JPAB,USPT,PGPB.	1312567
51S.DWPI,EPAB,JPAB,USPT,PGPB.	395
RAD51.DWPI,EPAB,JPAB,USPT,PGPB.	116
RAD51S	0
ANTISENS$	0
ANTISENS.DWPI,EPAB,JPAB,USPT,PGPB.	31
ANTISENSC.DWPI,EPAB,JPAB,USPT,PGPB.	8
ANTISENSE.DWPI,EPAB,JPAB,USPT,PGPB.	22985
ANTISENSEAND.DWPI,EPAB,JPAB,USPT,PGPB.	1
((RAD OR RAD 51 OR RAD51) SAME (ANTISENS$ OR RIBOYZM$)).USPT,PGPB,JPAB,EPAB,DWPI.	65

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Search Results - Record(s) 11 through 20 of 65 returned.

☐ 11. Document ID: US 20020058636 A1

L1: Entry 11 of 65 File: PGPB May 16, 2002

PGPUB-DOCUMENT-NUMBER: 20020058636
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020058636 A1

TITLE: RIBOZYMES TARGETING THE RETROVIRAL PACKAGING SEQUENCE EXPRESSION CONSTRUCTS AND RECOMBINANT RETROVIRUSES CONTAINING SUCH CONSTRUCTS

PUBLICATION-DATE: May 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
SYMONDS, GEOFFREY P.	ROSE BAY		AU	
SUN, LUN-QUAN	RYDE		AU	

US-CL-CURRENT: 514/44; 536/24.1, 536/24.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 12. Document ID: US 20020051730 A1

L1: Entry 12 of 65 File: PGPB May 2, 2002

PGPUB-DOCUMENT-NUMBER: 20020051730
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020051730 A1

TITLE: Coated medical devices and sterilization thereof

PUBLICATION-DATE: May 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bodnar, Stanko	Whitehouse Station	NJ	US	
Llanos, Gerard H.	Stewartsville	NJ	US	
Roller, Mark B.	North Brunswick	NJ	US	
Scopelianos, Angelo	Whitehouse Station	NJ	US	

US-CL-CURRENT: 422/33; 422/34

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 13. Document ID: US 20020032319 A1

L1: Entry 13 of 65 File: PGPB Mar 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020032319
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020032319 A1

TITLE: Human single nucleotide polymorphisms

PUBLICATION-DATE: March 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cargill, Michele	Gaithersburg	MD	US	
Ireland, James S.	Gaithersburg	MD	US	
Lander, Eric S.	Cambridge	MA	US	

US-CL-CURRENT: 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 14. Document ID: US 20020016625 A1

L1: Entry 14 of 65 File: PGPB Feb 7, 2002

PGPUB-DOCUMENT-NUMBER: 20020016625
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020016625 A1

TITLE: Drug/drug delivery systems for the prevention and treatment of vascular disease

PUBLICATION-DATE: February 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Falotico, Robert	Belle Mead	NJ	US	
Kopia, Gregory A.	Neshanic	NJ	US	
Llanos, Gerard H.	Stewartsville	NJ	US	
Siekierka, John	City Towaco	NJ	US	

US-CL-CURRENT: 623/1.13; 128/898, 623/1.38

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 15. Document ID: US 20020007215 A1

L1: Entry 15 of 65 File: PGPB Jan 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020007215

PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020007215 A1

TITLE: Drug/drug delivery systems for the prevention and treatment of vascular disease

PUBLICATION-DATE: January 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Falotico, Robert	Belle Mead	NJ	US	
Siekierka, John	Towaco	NJ	US	

US-CL-CURRENT: 623/1.21; 128/898, 623/1.15, 623/1.38

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 16. Document ID: US 20020007214 A1

L1: Entry 16 of 65 File: PGPB Jan 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020007214
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020007214 A1

TITLE: Drug/drug delivery systems for the prevention and treatment of vascular disease

PUBLICATION-DATE: January 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Falotico, Robert	Belle Mead	NJ	US	

US-CL-CURRENT: 623/1.21; 128/898, 623/1.38

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 17. Document ID: US 20020007213 A1

L1: Entry 17 of 65 File: PGPB Jan 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020007213
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020007213 A1

TITLE: Drug/drug delivery systems for the prevention and treatment of vascular disease

PUBLICATION-DATE: January 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Falotico, Robert	Belle Mead	NJ	US	
Kopia, Gregory A.	Hillsborough	NJ	US	
Llanos, Gerard H.	Stewartsville	NJ	US	
Sieklerka, John	Towaco	NJ	US	

US-CL-CURRENT: 623/1.21; 128/898, 623/1.38

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 18. Document ID: US 20020005206 A1

L1: Entry 18 of 65 File: PGPB Jan 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020005206
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020005206 A1

TITLE: Antiproliferative drug and delivery device

PUBLICATION-DATE: January 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Falotico, Robert	Belle Mead	NJ	US	
Kopia, Gregory A.	Hillsborough	NJ	US	
Llanos, Gerard H.	Stewartsville	NJ	US	
Siekjerka, John	Towaco	NJ	US	

US-CL-CURRENT: 128/898; 623/1.21, 623/1.38

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 19. Document ID: US 20010044145 A1

L1: Entry 19 of 65 File: PGPB Nov 22, 2001

PGPUB-DOCUMENT-NUMBER: 20010044145
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010044145 A1

TITLE: Methods of using mammalian RNase H and compositions thereof

PUBLICATION-DATE: November 22, 2001

INVENTOR-INFORMATION:

Term	Documents
RAD.DWPI,EPAB,JPAB,USPT,PGPB.	21036
RADS.DWPI,EPAB,JPAB,USPT,PGPB.	2562
"51".DWPI,EPAB,JPAB,USPT,PGPB.	1312567
51S.DWPI,EPAB,JPAB,USPT,PGPB.	395
RAD51.DWPI,EPAB,JPAB,USPT,PGPB.	116
RAD51S	0
ANTISENS$	0
ANTISENS.DWPI,EPAB,JPAB,USPT,PGPB.	31
ANTISENSC.DWPI,EPAB,JPAB,USPT,PGPB.	8
ANTISENSE.DWPI,EPAB,JPAB,USPT,PGPB.	22985
ANTISENSEAND.DWPI,EPAB,JPAB,USPT,PGPB.	1
((RAD OR RAD 51 OR RAD51) SAME (ANTISENS$ OR RIBOYZM$)).USPT,PGPB,JPAB,EPAB,DWPI.	65

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Search Results - Record(s) 21 through 30 of 65 returned.

☐ 21. Document ID: US 20010029351 A1

L1: Entry 21 of 65 File: PGPB Oct 11, 2001

PGPUB-DOCUMENT-NUMBER: 20010029351
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010029351 A1

TITLE: Drug combinations and delivery devices for the prevention and treatment of vascular disease

PUBLICATION-DATE: October 11, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Falotico, Robert	Belle Mead	NJ	US	
Kopia, Gregory A.	Hillsborough	NJ	US	
Landau, George	Verona	NJ	US	
Llanos, Gerard H.	Stewartsville	NJ	US	
Narayanan, Pallassana V.	Belle Mead	NJ	US	
Papandreou, George	Kendall Park	NJ	US	

US-CL-CURRENT: 604/103.02; 623/1.21

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 22. Document ID: US 6432655 B1

L1: Entry 22 of 65 File: USPT Aug 13, 2002

US-PAT-NO: 6432655
DOCUMENT-IDENTIFIER: US 6432655 B1

TITLE: Method of obtaining compositions

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 23. Document ID: US 6414025 B1

L1: Entry 23 of 65 File: USPT Jul 2, 2002

US-PAT-NO: 6414025
DOCUMENT-IDENTIFIER: US 6414025 B1

TITLE: Utilization of 2-hydroxy-4-trifluoromethylbenzoic acid derivatives as inhibitors of the activation of the nuclear transcription factors NF-.kappa..beta.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 24. Document ID: US 6406859 B1

L1: Entry 24 of 65 File: USPT Jun 18, 2002

US-PAT-NO: 6406859
DOCUMENT-IDENTIFIER: US 6406859 B1

TITLE: DNA encoding a 5-HT 1F receptor and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 25. Document ID: US 6376661 B1

L1: Entry 25 of 65 File: USPT Apr 23, 2002

US-PAT-NO: 6376661
DOCUMENT-IDENTIFIER: US 6376661 B1

TITLE: Human RNase H and compositions and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 26. Document ID: US 6368863 B1

L1: Entry 26 of 65 File: USPT Apr 9, 2002

US-PAT-NO: 6368863
DOCUMENT-IDENTIFIER: US 6368863 B1

TITLE: Reagents and methods for modulating gene expression through RNA mimicry

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 27. Document ID: US 6350868 B1

L1: Entry 27 of 65 File: USPT Feb 26, 2002

US-PAT-NO: 6350868
DOCUMENT-IDENTIFIER: US 6350868 B1

TITLE: Antisense human fucosyltransferase sequences and methods of use thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 28. Document ID: US 6338851 B1

L1: Entry 28 of 65 File: USPT Jan 15, 2002

US-PAT-NO: 6338851
DOCUMENT-IDENTIFIER: US 6338851 B1

TITLE: Method of suppressing an immune response to a transplanted organ or tissue by administering an OX-2 protein

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 29. Document ID: US 6335170 B1

L1: Entry 29 of 65 File: USPT Jan 1, 2002

US-PAT-NO: 6335170
DOCUMENT-IDENTIFIER: US 6335170 B1

TITLE: Gene expression in bladder tumors

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 30. Document ID: US 6326139 B1

L1: Entry 30 of 65 File: USPT Dec 4, 2001

US-PAT-NO: 6326139
DOCUMENT-IDENTIFIER: US 6326139 B1

TITLE: Method of screening for genetic predisposition to anticholinesterase therapy

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

Generate Collection | Print

Term	Documents
RAD.DWPI,EPAB,JPAB,USPT,PGPB.	21036
RADS.DWPI,EPAB,JPAB,USPT,PGPB.	2562
"51".DWPI,EPAB,JPAB,USPT,PGPB.	1312567
51S.DWPI,EPAB,JPAB,USPT,PGPB.	395
RAD51.DWPI,EPAB,JPAB,USPT,PGPB.	116
RAD51S	0
ANTISENS$	0
ANTISENS.DWPI,EPAB,JPAB,USPT,PGPB.	31
ANTISENSC.DWPI,EPAB,JPAB,USPT,PGPB.	8
ANTISENSE.DWPI,EPAB,JPAB,USPT,PGPB.	22985
ANTISENSEAND.DWPI,EPAB,JPAB,USPT,PGPB.	1
((RAD OR RAD 51 OR RAD51) SAME (ANTISENS$ OR RIBOYZM$)).USPT,PGPB,JPAB,EPAB,DWPI.	65

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☐ 31. Document ID: US 6245523 B1

L1: Entry 31 of 65 File: USPT Jun 12, 2001

US-PAT-NO: 6245523
DOCUMENT-IDENTIFIER: US 6245523 B1

TITLE: Survivin, a protein that inhibits cellular apoptosis, and its modulation

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 32. Document ID: US 6232529 B1

L1: Entry 32 of 65 File: USPT May 15, 2001

US-PAT-NO: 6232529
DOCUMENT-IDENTIFIER: US 6232529 B1

TITLE: Methods of producing high-oil seed by modification of starch levels

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 33. Document ID: US 6225115 B1

L1: Entry 33 of 65 File: USPT May 1, 2001

US-PAT-NO: 6225115
DOCUMENT-IDENTIFIER: US 6225115 B1

TITLE: DNA encoding taurine and GABA transporters and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 34. Document ID: US 6114167 A

L1: Entry 34 of 65 File: USPT Sep 5, 2000

US-PAT-NO: 6114167
DOCUMENT-IDENTIFIER: US 6114167 A

TITLE: Ribozymes targeting the MoMLV PSI packaging sequence and the HIV tat sequence

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 39. Document ID: US 6027892 A

L1: Entry 39 of 65 File: USPT Feb 22, 2000

US-PAT-NO: 6027892
DOCUMENT-IDENTIFIER: US 6027892 A

TITLE: Compositions and methods for reducing radiation and drug resistance in cells

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 40. Document ID: US 6018042 A

L1: Entry 40 of 65 File: USPT Jan 25, 2000

US-PAT-NO: 6018042
DOCUMENT-IDENTIFIER: US 6018042 A

TITLE: Antitumor antisense oligonucleotides

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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Term	Documents
RAD.DWPI,EPAB,JPAB,USPT,PGPB.	21036
RADS.DWPI,EPAB,JPAB,USPT,PGPB.	2562
"51".DWPI,EPAB,JPAB,USPT,PGPB.	1312567
51S.DWPI,EPAB,JPAB,USPT,PGPB.	395
RAD51.DWPI,EPAB,JPAB,USPT,PGPB.	116
RAD51S	0
ANTISENS$	0
ANTISENS.DWPI,EPAB,JPAB,USPT,PGPB.	31
ANTISENSC.DWPI,EPAB,JPAB,USPT,PGPB.	8
ANTISENSE.DWPI,EPAB,JPAB,USPT,PGPB.	22985
ANTISENSEAND.DWPI,EPAB,JPAB,USPT,PGPB.	1
((RAD OR RAD 51 OR RAD51) SAME (ANTISENS$ OR RIBOYZM$)).USPT,PGPB,JPAB,EPAB,DWPI.	65

There are more results than shown above. Click here to view the entire set.

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Search Results - Record(s) 41 through 50 of 65 returned.

☐ 41. Document ID: US 6011200 A

L1: Entry 41 of 65 File: USPT Jan 4, 2000

US-PAT-NO: 6011200
DOCUMENT-IDENTIFIER: US 6011200 A

TITLE: Methods for altering the rate of plant development and plants obtained therefrom

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image | KWIC

☐ 42. Document ID: US 6001653 A

L1: Entry 42 of 65 File: USPT Dec 14, 1999

US-PAT-NO: 6001653
DOCUMENT-IDENTIFIER: US 6001653 A

TITLE: Human type 2 RNase H

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image | KWIC

☐ 43. Document ID: US 5998187 A

L1: Entry 43 of 65 File: USPT Dec 7, 1999

US-PAT-NO: 5998187
DOCUMENT-IDENTIFIER: US 5998187 A

TITLE: Receptor tyrosine kinase

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image | KWIC

☐ 44. Document ID: US 5990299 A

L1: Entry 44 of 65 File: USPT Nov 23, 1999

US-PAT-NO: 5990299
DOCUMENT-IDENTIFIER: US 5990299 A

TITLE: Control of CD44 gene expression for therapeutic use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 45. Document ID: US 5985585 A

L1: Entry 45 of 65 File: USPT Nov 16, 1999

US-PAT-NO: 5985585
DOCUMENT-IDENTIFIER: US 5985585 A

TITLE: Processes using a human serotonin receptor (5-HT.sub.4B)

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 46. Document ID: US 5965439 A

L1: Entry 46 of 65 File: USPT Oct 12, 1999

US-PAT-NO: 5965439
DOCUMENT-IDENTIFIER: US 5965439 A

TITLE: Host defense enhancement

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 47. Document ID: US 5945339 A

L1: Entry 47 of 65 File: USPT Aug 31, 1999

US-PAT-NO: 5945339
DOCUMENT-IDENTIFIER: US 5945339 A

TITLE: Methods to promote homologous recombination in eukaryotic cells and organisms

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 48. Document ID: US 5914449 A

L1: Entry 48 of 65 File: USPT Jun 22, 1999

US-PAT-NO: 5914449
DOCUMENT-IDENTIFIER: US 5914449 A

TITLE: Method for increasing storage lipid content in plant seed

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 49. Document ID: US 5874564 A

L1: Entry 49 of 65 File: USPT Feb 23, 1999

US-PAT-NO: 5874564
DOCUMENT-IDENTIFIER: US 5874564 A

TITLE: Reagents and methods for modulating gene expression through RNA mimicry

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 50. Document ID: US 5856094 A

L1: Entry 50 of 65 File: USPT Jan 5, 1999

US-PAT-NO: 5856094
DOCUMENT-IDENTIFIER: US 5856094 A

TITLE: Method of detection of neoplastic cells

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

Generate Collection | Print

Term	Documents
RAD.DWPI,EPAB,JPAB,USPT,PGPB.	21036
RADS.DWPI,EPAB,JPAB,USPT,PGPB.	2562
"51".DWPI,EPAB,JPAB,USPT,PGPB.	1312567
51S.DWPI,EPAB,JPAB,USPT,PGPB.	395
RAD51.DWPI,EPAB,JPAB,USPT,PGPB.	116
RAD51S	0
ANTISENS$	0
ANTISENS.DWPI,EPAB,JPAB,USPT,PGPB.	31
ANTISENSC.DWPI,EPAB,JPAB,USPT,PGPB.	8
ANTISENSE.DWPI,EPAB,JPAB,USPT,PGPB.	22985
ANTISENSEAND.DWPI,EPAB,JPAB,USPT,PGPB.	1
((RAD OR RAD 51 OR RAD51) SAME (ANTISENS$ OR RIBOYZM$)).USPT,PGPB,JPAB,EPAB,DWPI.	65

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Search Results - Record(s) 51 through 60 of 65 returned.

☐ 51. Document ID: US 5807671 A

L1: Entry 51 of 65 File: USPT Sep 15, 1998

US-PAT-NO: 5807671
DOCUMENT-IDENTIFIER: US 5807671 A

TITLE: Method of screening for genetic predisposition to anticholinesterase therapy

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 52. Document ID: US 5780296 A

L1: Entry 52 of 65 File: USPT Jul 14, 1998

US-PAT-NO: 5780296
DOCUMENT-IDENTIFIER: US 5780296 A

TITLE: Compositions and methods to promote homologous recombination in eukaryotic cells and organisms

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 53. Document ID: US 5766848 A

L1: Entry 53 of 65 File: USPT Jun 16, 1998

US-PAT-NO: 5766848
DOCUMENT-IDENTIFIER: US 5766848 A

TITLE: Methods for identifying compounds which specifically bind a human betaine/GABA transporter

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 54. Document ID: US 5736294 A

L1: Entry 54 of 65 File: USPT Apr 7, 1998

US-PAT-NO: 5736294
DOCUMENT-IDENTIFIER: US 5736294 A

TITLE: Reagents and methods for modulating gene expression through RNA mimicry

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 55. Document ID: US 5712384 A

L1: Entry 55 of 65 File: USPT Jan 27, 1998

US-PAT-NO: 5712384
DOCUMENT-IDENTIFIER: US 5712384 A

TITLE: Ribozymes targeting retroviral packaging sequence expression constructs and recombinant retroviruses containing such constructs

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 56. Document ID: US 5681714 A

L1: Entry 56 of 65 File: USPT Oct 28, 1997

US-PAT-NO: 5681714
DOCUMENT-IDENTIFIER: US 5681714 A

TITLE: Nucleic acid encoding tek receptor tyrosine kinase

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 57. Document ID: US 5639652 A

L1: Entry 57 of 65 File: USPT Jun 17, 1997

US-PAT-NO: 5639652
DOCUMENT-IDENTIFIER: US 5639652 A

TITLE: DNA encoding a human 5-HT.sub.1F receptor and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 58. Document ID: US 5583034 A

L1: Entry 58 of 65 File: USPT Dec 10, 1996

US-PAT-NO: 5583034
DOCUMENT-IDENTIFIER: US 5583034 A

TITLE: Enhancement of adoptosis using antisense oligonucleotides

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 59. Document ID: US 5508179 A

L1: Entry 59 of 65 File: USPT Apr 16, 1996

US-PAT-NO: 5508179
DOCUMENT-IDENTIFIER: US 5508179 A

TITLE: Use of deoxyribose nicotinamide adenine dinucleotide to enhance the specificity of NAD.sup.+ -dependent ligation reactions

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 60. Document ID: US 5466596 A

L1: Entry 60 of 65 File: USPT Nov 14, 1995

US-PAT-NO: 5466596
DOCUMENT-IDENTIFIER: US 5466596 A

TITLE: Tissue specific transcriptional regulatory element

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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Term	Documents
RAD.DWPI,EPAB,JPAB,USPT,PGPB.	21036
RADS.DWPI,EPAB,JPAB,USPT,PGPB.	2562
"51".DWPI,EPAB,JPAB,USPT,PGPB.	1312567
51S.DWPI,EPAB,JPAB,USPT,PGPB.	395
RAD51.DWPI,EPAB,JPAB,USPT,PGPB.	116
RAD51S	0
ANTISENS$	0
ANTISENS.DWPI,EPAB,JPAB,USPT,PGPB.	31
ANTISENSC.DWPI,EPAB,JPAB,USPT,PGPB.	8
ANTISENSE.DWPI,EPAB,JPAB,USPT,PGPB.	22985
ANTISENSEAND.DWPI,EPAB,JPAB,USPT,PGPB.	1
((RAD OR RAD 51 OR RAD51) SAME (ANTISENS$ OR RIBOYZM$)).USPT,PGPB,JPAB,EPAB,DWPI.	65

There are more results than shown above. Click here to view the entire set.

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Search Results - Record(s) 61 through 65 of 65 returned.

☐ 61. Document ID: US 5248671 A

L1: Entry 61 of 65 File: USPT Sep 28, 1993

US-PAT-NO: 5248671
DOCUMENT-IDENTIFIER: US 5248671 A

TITLE: Methods and compositions for treatment of cancer using oligonucleotides

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 62. Document ID: US 5087617 A

L1: Entry 62 of 65 File: USPT Feb 11, 1992

US-PAT-NO: 5087617
DOCUMENT-IDENTIFIER: US 5087617 A

TITLE: Methods and compositions for treatment of cancer using oligonucleotides

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 63. Document ID: WO 200053630 A2 AU 200037331 A

L1: Entry 63 of 65 File: DWPI Sep 14, 2000

DERWENT-ACC-NO: 2000-594307
DERWENT-WEEK: 200056
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TITLE: Peptides capable of binding to mammalian Rad51, used to treat hyperproliferative disorders in mammals, especially humans, and to screen for other DNA break repair disrupters

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 64. Document ID: WO 200047231 A2 AU 200028696 A EP 1150691 A2

L1: Entry 64 of 65 File: DWPI Aug 17, 2000

DERWENT-ACC-NO: 2000-506091

DERWENT-WEEK: 200045
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TITLE: Inhibiting cell proliferation useful for cancer therapy, comprises administering Rad51 inhibitor in vivo

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 65. Document ID: AU 730220 B WO 9709433 A1 AU 9668846 A NO 9800957 A EP 856058 A1 BR 9610168 A CN 1201492 A JP 11511984 W CZ 9800656 A3 HU 9903075 A2 MX 9801699 A1 SK 9800302 A3

L1: Entry 65 of 65 File: DWPI Mar 1, 2001

DERWENT-ACC-NO: 1997-192908
DERWENT-WEEK: 200117
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TITLE: Human ataxia and rad related checkpoint protein - and yeast homologue Rad3, controls progression through cell cycle and can screen for checkpoint mechanism abrogators, useful in cancer treatment

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

Generate Collection | Print

Term	Documents
RAD.DWPI,EPAB,JPAB,USPT,PGPB.	21036
RADS.DWPI,EPAB,JPAB,USPT,PGPB.	2562
"51".DWPI,EPAB,JPAB,USPT,PGPB.	1312567
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RAD51S	0
ANTISENS$	0
ANTISENS.DWPI,EPAB,JPAB,USPT,PGPB.	31
ANTISENSC.DWPI,EPAB,JPAB,USPT,PGPB.	8
ANTISENSE.DWPI,EPAB,JPAB,USPT,PGPB.	22985
((RAD OR RAD 51 OR RAD51) SAME (ANTISENS$ OR RIBOYZM$)).USPT,PGPB,JPAB,EPAB,DWPI.	65

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Display Format: - Change Format

ILIGHT set on as ' '
? b 155, 5
       25aug02 15:03:23 User242957 Session D484.2
            $0.00   0.070 DialUnits File410
     $0.00 Estimated cost File410
     $0.00 Estimated cost this search
     $0.00 Estimated total session cost   0.215 DialUnits

SYSTEM:OS - DIALOG OneSearch
  File 155:MEDLINE(R) 1966-2002/Aug W3
*File 155: Alert feature enhanced for multiple files, duplicates removal, customized scheduling. See HELP ALERT.
  File   5:Biosis Previews(R) 1969-2002/Aug W2
         (c) 2002 BIOSIS
*File   5: Alert feature enhanced for multiple files, duplicates removal, customized scheduling. See HELP ALERT.

      Set Items Description
      --- ----- -----------
? s rad and 51 or rad51
          12769 RAD
         173108 51
           1269 RAD51
      S1   1461 RAD AND 51 OR RAD51
? s s1 and antisens?
           1461 S1
          35063 ANTISENS?
      S2   18 S1 AND ANTISENS?
? rd
...completed examining records
      S3   11 RD (unique items)
? s s1 and riboyzm?
           1461 S1
              0 RIBOYZM?
      S4   0 S1 AND RIBOYZM?
? t s3/3,ab/all

 3/3,AB/1   (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13105761   21659790   PMID: 11801733
   Formation of higher-order nuclear **Rad51** structures is functionally linked to p21 expression and protection from DNA damage-induced apoptosis.
  Raderschall Elke; Bazarov Alex; Cao Jiangping; Lurz Rudi; Smith Avril; Mann Wolfgang; Ropers Hans-Hilger; Sedivy John M; Golub Efim I; Fritz Eberhard; Haaf Thomas
  Max Planck Institute of Molecular Genetics, 14195 Berlin, Germany.
  Journal of cell science (England) Jan 1 2002, 115 (Pt 1) p153-64,
ISSN 0021-9533 Journal Code: 0052457
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  After exposure of mammalian cells to DNA damage, the endogenous **Rad51** recombination protein is concentrated in multiple discrete foci, which are thought to represent nuclear domains for recombinational DNA repair. Overexpressed **Rad51** protein forms foci and higher-order nuclear structures, even in the absence of DNA damage, in cells that do not undergo DNA replication synthesis. This correlates with increased expression of the cyclin-dependent kinase (Cdk) inhibitor p21. Following DNA damage, constitutively **Rad51**-overexpressing cells show reduced numbers of DNA breaks and chromatid-type chromosome aberrations and a greater resistance to apoptosis. In contrast, **Rad51 antisense** inhibition reduces p21 protein levels and sensitizes cells to etoposide

treatment. Downregulation of p21 inhibits **Rad51** foci formation in both normal and **Rad51**-overexpressing cells. Collectively, our results show that **Rad51** expression, **Rad51** foci formation and p21 expression are interrelated, suggesting a functional link between mammalian **Rad51** protein and p21-mediated cell cycle regulation. This mechanism may contribute to a highly effective recombinational DNA repair in cell cycle-arrested cells and protection against DNA damage-induced apoptosis.

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12869845 21626115 PMID: 11754170
Expression of base excision, mismatch, and recombination repair genes in the organogenesis-stage rat conceptus and effects of exposure to a genotoxic teratogen, 4-hydroperoxycyclophosphamide.
Vinson R K; Hales B F
Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada, H3G 1Y6.
Teratology (United States) Dec 2001, 64 (6) p283-91, ISSN 0040-3709 Journal Code: 0153257
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: DNA repair capability may influence the outcome of genotoxic teratogen exposure. The goals of this study were to assess the expression of base excision repair (BER), mismatch repair (MMR), and recombination repair (RCR) genes in the mid-organogenesis rat conceptus and to determine the effects on expression of exposure to the genotoxic teratogen, 4-hydroperoxycyclophosphamide (4-OOHCPA). METHODS: The expression of 17 BER, MMR, and RCR genes was examined in gestational day (GD) 10-12 rat conceptuses using the **antisense** RNA (aRNA) technique. Embryos were cultured with 10 microM 4-OOHCPA to examine effects on gene expression. RESULTS: Yolk sacs and embryos had similar gene expression patterns for all three DNA repair pathways from GD10-12. Transcripts for APNG, PMS1, and RAD54 were present at high concentrations in both tissues. The remainder of the genes were expressed at low levels in yolk sac, with a few not detected on GD10 and 11. In the embryo, transcripts for most genes were low on GD10 and 11; several increased by GD12. After exposure to 4-OOHCPA for 24 hr, XRCC1 and RAD57 expression decreased in yolk sac, whereas only **RAD51** transcripts decreased in the embryo. By 44 hr, transcripts for all BER genes decreased in yolk sac; in the embryo, most BER, MMR, and RCR genes decreased, many below the level of detection. CONCLUSIONS: The expression of DNA repair genes in the mid-organogenesis rat conceptus is varied and subject to down-regulation by 4-OOHCPA. DNA repair gene expression may determine the consequences of genotoxicant exposure during development.

3/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09805408 98238634 PMID: 9571148
In vitro and in vivo potentiation of radiosensitivity of malignant gliomas by **antisense** inhibition of the **RAD51** gene.
Ohnishi T; Taki T; Hiraga S; Arita N; Morita T
Department of Neurosurgery, Osaka University Medical School, Japan. ohnishi@nsurg.med.osaka-u.ac.jp
Biochemical and biophysical research communications (UNITED STATES) Apr 17 1998, 245 (2) p319-24, ISSN 0006-291X Journal Code: 0372516
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed

The mammalian **RAD51** gene is a homologue of the yeast **RAD51** and E. coli RecA genes, which are related to the repair of DNA double-strand breaks and are also involved in recombination repair and various SOS responses to DNA damage by gamma-irradiation and alkylating reagents. In this study, we investigated both in vitro and in vivo whether inhibition of the **RAD51** gene by **antisense** oligonucleotides (ODNs) enhances the radiosensitivity of mouse malignant gliomas. A volume of 100 nM of **RAD51 antisense** ODNs inhibited the level of mRNA by more than 95% and reduced the protein expression by about 70%. Treatment of mouse 203G glioma cells with 100 nM of **RAD51 antisense** ODNs significantly enhanced the radiation-induced cell kill compared to control cells, and cells treated with sense or scrambled ODNs. When the glioma cells were implanted in the cisterna magna of mice followed by treatment with **RAD51 antisense** ODNs, the survival time of the mice was markedly prolonged compared to that of the untreated group ($p < 0.001$, logrank test). In addition, the combination of **antisense** ODNs and irradiation extended the survival time of the glioma-bearing mice much longer than could be achieved with radiation alone ($p < 0.0001$, logrank test). These results suggest that inhibition of **RAD51** can be expected to serve as a novel potentiator for radiation therapy in malignant gliomas by inhibiting DNA double-strand break repair.

3/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09740240 98175545 PMID: 9515792

BRCA1 up-regulation is associated with repair-mediated resistance to cis-diamminedichloroplatinum(II).

Husain A; He G; Venkatraman E S; Spriggs D R

Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA.

Cancer research (UNITED STATES) Mar 15 1998, 58 (6) p1120-3, ISSN 0008-5472 Journal Code: 2984705R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We sought to identify novel genes associated with cis-diamminedichloroplatinum(II) (CDDP) resistance, and by differential display analysis, we found that the human breast and ovarian cancer susceptibility gene BRCA1 was overexpressed in CDDP-resistant MCF-7 cells. A recent report that BRCA1 and human **Rad51** colocalize in S-phase cells suggests a role for BRCA1 in DNA damage repair. We hypothesized that BRCA1 plays a role in DNA damage repair-mediated CDDP resistance. In CCDP-resistant variants of breast and ovarian carcinoma cell lines, MCF-7 CDDP/R and SKOV-3 CDDP/R, we found increased levels of BRCA1 protein, and we determined that the SKOV-3 CDDP/R cell line is significantly more proficient at DNA damage repair. **Antisense** inhibition of BRCA1 in this cell line resulted in an increased sensitivity to CDDP, a decreased proficiency of DNA repair, and an enhanced rate of apoptosis. These data support the hypothesis that BRCA1 is a gene involved in DNA damage repair.

3/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09620461 98038784 PMID: 9372947

Elevated recombination in immortal human cells is mediated by HsRAD51 recombinase.

Xia S J; Shammas M A; Shmookler Reis R J

Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock 72205, USA.

Molecular and cellular biology (UNITED STATES) Dec 1997, 17 (12) p7151-8, ISSN 0270-7306 Journal Code: 8109087
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Normal diploid cells have a limited replicative potential in culture, with progressively increasing interdivision time. Rarely, cell lines arise which can divide indefinitely; like tumor cells, such "immortal" lines display frequent chromosomal aberrations which may reflect high rates of recombination. Recombination frequencies within a plasmid substrate were 3.5-fold higher in nine immortal human cell lines than in six untransformed cell strains. Expression of HsRAD51, a human homolog of the yeast **RAD51** and Escherichia coli recA recombinase genes, was 4.5-fold higher in immortal cell lines than in mortal cells. Stable transformation of human fibroblasts with simian virus 40 large T antigen prior to cell immortalization increased both chromosomal recombination and the level of HsRAD51 transcripts by two- to fivefold. T-antigen induction of recombination was efficiently blocked by introduction of HsRAD51 **antisense** (but not control) oligonucleotides spanning the initiation codon, implying that HsRAD51 expression mediates augmented recombination. Since p53 binds and inactivates HsRAD51, T-antigen-p53 association may block such inactivation and liberate HsRAD51. Upregulation of HsRAD51 transcripts in T-antigen-transformed and other immortal cells suggests that recombinase activation can also occur at the RNA level and may facilitate cell transformation to immortality.

3/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08903269 96264658 PMID: 8670299
Antisense inhibition of the **RAD51** enhances radiosensitivity.
Taki T; Ohnishi T; Yamamoto A; Hiraga S; Arita N; Izumoto S; Hayakawa T; Morita T
Department of Neurosurgery, Osaka University Medical School, Japan.
Biochemical and biophysical research communications (UNITED STATES) Jun 14 1996, 223 (2) p434-8, ISSN 0006-291X Journal Code: 0372516
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The mammalian **RAD51** gene is a homologue of the yeast **RAD51** and E. coli RecA genes, which are involved in recombination and DNA repair. We examined the role of **RAD51** protein in mouse cells using **RAD51 antisense** phosphorothioate oligonucleotides (ODNs). The extraluminal delivery of 50 nM or 100 nM of **antisense** ODNs with lipofection to mouse cells resulted 90% suppression of **RAD51** protein expression. The **antisense** ODNs significantly inhibited the cell growth and the treated cells became more sensitive to gamma-irradiation than the control groups. These results indicate mouse **RAD51** plays an essential role in cell proliferation and radioresistant activity.

3/3,AB/7 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13780469 BIOSIS NO.: 200200409290
Rad51 inhibitors sensitize human tumor cells to DNA damaging treatment with doxorubicin or cisplatin.
AUTHOR: Vallerga Anne K(a); Yang Jerry(a); Reddy Guru(a); Zarling David A (a)
AUTHOR ADDRESS: (a)Pangene Corporation, Fremont, CA**USA

JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 43p795 March, 2002
MEDIUM: print
CONFERENCE/MEETING: 93rd Annual Meeting of the American Association for Cancer Research San Francisco, California, USA April 06-10, 2002
ISSN: 0197-016X
RECORD TYPE: Citation
LANGUAGE: English
2002

3/3,AB/8 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13660030 BIOSIS NO.: 200200288851
The transcriptional inhibition of DNA repair protein **Rad51** enhances radiosensitivity in prostate cancer cells.
AUTHOR: Nishimura H(a); Sasaki R(a); Soejima T(a); Ejima Y(a); Yoden E(a); Shirakawa T; Ota Y(a); Matsumoto A; Sugimura K(a)
AUTHOR ADDRESS: (a)Radiology, Kobe University, Kobe**Japan
JOURNAL: European Journal of Cancer 37 (Supplement 6):pS143 October, 2001
MEDIUM: print
CONFERENCE/MEETING: 11th European Cancer Conference Lisbon, Portugal October 21-25, 2001
ISSN: 0959-8049
RECORD TYPE: Citation
LANGUAGE: English
2001

3/3,AB/9 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13647466 BIOSIS NO.: 200200276287
The transcriptional inhibition of DNA repair protein **Rad51** enhances radiosensitivity in prostate cancer cells.
AUTHOR: Sasaki R(a); Nishimura H(a); Soejima T(a); Ejima Y(a); Yoden E(a); Shirakawa T; Gotoh A; Ota Y(a); Matsumoto A; Sugimura K(a)
AUTHOR ADDRESS: (a)Radiology, Kobe University School of Medicine, Kobe** Japan
JOURNAL: International Journal of Radiation Oncology Biology Physics 51 (3 Supplement 1):p56-57 2001
MEDIUM: print
CONFERENCE/MEETING: 43rd Annual Meeting of the American Society for Therapeutic Radiology and Oncology San Francisco, CA, USA November 04-08, 2001
ISSN: 0360-3016
RECORD TYPE: Citation
LANGUAGE: English
2001

3/3,AB/10 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13115199 BIOSIS NO.: 200100322348
Oncogenic tyrosine kinases derived from chromosomal translocations induce common mechanisms of drug resistance: Overexpression of **RAD51** and G2/M arrest.
AUTHOR: Slupianek A(a); Hoser G; Fishel R; Skorski T
AUTHOR ADDRESS: (a)Temple University, Center for Biotechnology, Thomas

Jefferson University, Kimmel Cancer Center, Philadelphia, PA**USA
JOURNAL: Blood 96 (11 Part 1):p825a November 16, 2000
MEDIUM: print
CONFERENCE/MEETING: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000
SPONSOR: American Society of Hematology
ISSN: 0006-4971
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Oncogenic tyrosine kinases (OTKs) such as BCR/ABL, TEL/ABL, TEL/JAK2, TEL/PDGFR and NPM/ALK arose from the chromosomal translocations induce acute and chronic myelogenous leukemias or non-Hodgkin's lymphoma. Hematopoietic cell lines transformed by these OTKs are resistant to DNA damaging drugs such as cisplatin and mitomycin. Treatment with one of these drugs induce transient G2/M arrest in cells transformed by OTKs, but not in normal cells. G2/M arrest appears to be essential for drug resistance in the former cells. In addition, OTK-transformed cells display elevated levels of **RAD51**, a protein involved in reparation of drug-induced DNA lesions. **RAD51** is a member of conserved family of eukaryotic proteins related to Escherichia coli RecA protein, which plays a central role in prokaryotic response to DNA damage. Both, RecA and **RAD51** promote homology-dependent repair of double strand breaks (DSBs), probably the most disruptive type of lesion in DNA after exposure to DNA-damaging agents. If left unrepaired, DSBs lead to broken chromosomes and cell death. OTKs-induced elevation of **RAD51** expression is probably due to the STAT5-dependent transactivation of **RAD51** promoter. Using the in vivo DSBs repair model in which DSBs are induced in the green fluorescent protein (GFP) sequence and their reparation is assessed by the appearance of GFP+ cells, we found that **RAD51** is responsible for enhanced DSBs repair in OTKs-transformed cells. Downregulation of **RAD51** expression by the **antisense** cDNA reduced almost completely drug resistance in the transformed cells. Since **RAD51**-dependent DSBs repair occurs usually in the late S phase and/or early G2/M phase we postulate that elevated expression of **RAD51** in combination with G2/M arrest is responsible for more efficient repair of drug-induced lethal DNA lesions in OTKs-transformed cells in comparison to normal cells.

2000

3/3,AB/11 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13105365 BIOSIS NO.: 200100312514
Functional link of BCR/ABL oncogenic tyrosine kinase and **RAD51** double strand break repair protein in DNA damage response.
AUTHOR: Slupianek A(a); Tombline G; Schmutte C; Nieborowska-Skorska M; Malecki M; Fishel R; Skorski T
AUTHOR ADDRESS: (a)Center for Biotechnology, Temple University, Philadelphia, PA**USA
JOURNAL: Blood 96 (11 Part 1):p509a November 16, 2000
MEDIUM: print
CONFERENCE/MEETING: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000
SPONSOR: American Society of Hematology
ISSN: 0006-4971
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Double-strand breaks (DSBs), probably the most disruptive type of lesion in DNA, may arise after exposure to DNA-damaging agents. If left unrepaired, DSBs lead to broken chromosomes and cell death. Philadelphia chromosome-positive (Ph1) leukemias expressing BCR/ABL oncogenic tyrosine kinases are usually resistant to DNA damaging agents (cytostatics, radiation) inducing DSBs. Using representational differences analysis (RDA) followed by Northern blotting and Western blotting we found that BCR/ABL kinase induces overexpression of **RAD51** in hematopoietic cell lines and in chronic myelogenous leukemia (CML) cells. **RAD51** is a member of conserved family of eukaryotic proteins related to Escherichia coli RecA protein, which plays a central role in prokaryotic response to DNA damage. Both, RecA and **RAD51** promote homology-dependent repair of DSBs. BCR/ABL-induced elevation of **RAD51** expression is due to the STAT5-mediated transactivation of **RAD51** promoter and the prevention of **RAD51** cleavage by inhibition of caspase-3. BCR/ABL is in complex with **RAD51** and induces its phosphorylation on Y315, which increases **RAD51** cytoplasmic-nuclear shuttling and assembly on DNA lesions (DSBs). Using the in vivo DSBs repair model in which DSBs are induced in the green fluorescent protein (GFP) sequence and their reparation is assessed by the appearance of GFP+ cells, we found that **RAD51** is responsible for enhanced DSBs repair in BCR/ABL-transformed cells. Inhibition of **RAD51** expression and/or function by the **antisense** cDNA or the Y315F mutant reduced almost completely drug resistance in BCR/ABL-transformed cells. Incubation of BCR/ABL-positive cells with the ABL kinase inhibitor STI571 caused downregulation of expression of **RAD51** and abrogated drug resistance. Expression of exogenous **RAD51** elevated the total amount of **RAD51** protein and partially rescued drug resistance in these cells. In contrast to drug-induced apoptosis, modulation of **RAD51** expression did not affect the susceptibility of normal and BCR/ABL-transformed cells to apoptosis induced by growth factor withdrawal. Moreover, **RAD51** does not seem to be directly involved in regulation of G2/M cell cycle phase, P-glycoprotein or caspase-3, which may be involved in drug resistance. Instead, BCR/ABL-dependent overexpression of **RAD51** is responsible for enhanced reparation of drug-induced lethal DNA lesions (DSBs), which decrease activation/accumulation of the "DNA damage sensor" p73 and reduce the pro-apoptotic signaling from the nucleus. Thus, BCR/ABL-induced and **RAD51**-mediated DNA repair represents a novel mechanism contributing to drug resistance in Ph1 leukemias.

2000
? e au=ohnishi takanori

Ref	Items	Index-term
E1	9	AU=OHNISHI TAKAMASA
E2	10	AU=OHNISHI TAKAMITSU
E3	28	*AU=OHNISHI TAKANORI
E4	44	AU=OHNISHI TAKASHI
E5	4	AU=OHNISHI TAKAYA
E6	5	AU=OHNISHI TAKAYUKI
E7	5	AU=OHNISHI TAKENAO
E8	93	AU=OHNISHI TAKEO
E9	1	AU=OHNISHI TAKUJI
E10	1	AU=OHNISHI TATSUJI
E11	5	AU=OHNISHI TEIJI
E12	20	AU=OHNISHI TETSUO

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S5 28 AU='OHNISHI TAKANORI'
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Ref	Items	Index-term
E1	1	AU=OHNISHI SUN-ICHI
E2	1	AU=OHNISHI SYUNSUKE
E3	1360	*AU=OHNISHI T
E4	14	AU=OHNISHI TADASHI
E5	20	AU=OHNISHI TAIRA
E6	1	AU=OHNISHI TAKAEO
E7	3	AU=OHNISHI TAKAFUMI
E8	16	AU=OHNISHI TAKAHIRO
E9	2	AU=OHNISHI TAKAKO
E10	9	AU=OHNISHI TAKAMASA
E11	10	AU=OHNISHI TAKAMITSU
E12	28	AU=OHNISHI TAKANORI

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1360 AU=OHNISHI T
28 AU=OHNISHI TAKANORI
S6 1388 AU='OHNISHI T' OR AU='OHNISHI TAKANORI'
? s s6 ir s5
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? s s6 or s5
1388 S6
28 S5
S7 1388 S6 OR S5
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1388 S7
12769 RAD
S8 1 S7 AND RAD
? t s8/3,ab/all

8/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

04728282 BIOSIS NO.: 000080031408
CHARACTERISTICS OF DNA REPAIR OF A UV-SENSITIVE MUTANT **RAD**-C OF DICTYOSTELIUM-DISCOIDEUM
AUTHOR: TANO K; **OHNISHI T**; SATO N; NOZU K
AUTHOR ADDRESS: DEP. BIOCHEM., NARA MED. UNIV., KASHIHARA, NARA 634, JPN.
JOURNAL: MOL GEN GENET 198 (3). 1985. 385-389. 1985
FULL JOURNAL NAME: Molecular & General Genetics

CODEN: MGGEA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A radiation-sensitive mutant, TW8(radC), of D. discoideum is more sensitive to UV light killing than the parental wild strain NC4(**RAD** -), but is resistant to 4-nitroquinoline-1-oxide (4NQO) at almost the same level as NC4. In TW8 amoebae, single-strand breaks of DNA molecules were hardly detectable immediately after UV irradiation, and the removal of pyrimidine dimers was depressed during the postirradiation incubation when compared with that of NC4 amoebae. After treatment with 4NQO, however, single-strand breaks were detected in TW8 amoebae. The almost complete rejoining of these breaks was also detected after the removal of 4HAQO-adducts. The TW8 amoebae have an efficient repair capacity against DNA damage caused by 4NQO, MMS [methyl methanesulfonate], MMC [mitomycin C] and MNNG [N-methyl-N'-nitro-N-nitrosoguanidine] but not UV.

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Set	Items	Description
S1	1461	RAD AND 51 OR RAD51
S2	18	S1 AND ANTISENS?
S3	11	RD (unique items)
S4	0	S1 AND RIBOYZM?
S5	28	AU='OHNISHI TAKANORI'
S6	1388	AU='OHNISHI T' OR AU='OHNISHI TAKANORI'
S7	1388	S6 OR S5
S8	1	S7 AND RAD

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? s s7 and (antisens? or riboyzm?)
            1388  S7
           35063  ANTISENS?
               0  RIBOYZM?
      S9       5  S7 AND (ANTISENS? OR RIBOYZM?)
? rd
...completed examining records
     S10       3  RD (unique items)
? t s10/3,ab/all

 10/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10065175   99048960   PMID: 9832120
  Steroidogenic acute regulatory protein (StAR) transcripts constitutively
expressed in the adult rat central nervous system: colocalization of StAR,
cytochrome P-450SCC (CYP XIA1), and 3beta-hydroxysteroid dehydrogenase in
the rat brain.
  Furukawa A; Miyatake A; Ohnishi T; Ichikawa Y
  Department of Biochemistry, Faculty of Medicine, Kagawa Medical
University, Japan.
  Journal of neurochemistry (UNITED STATES) Dec 1998, 71 (6) p2231-8,
ISSN 0022-3042 Journal Code: 2985190R
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  Steroidogenic acute regulatory protein (StAR) is a 30-kDa protein
involved in the transport of cholesterol to the inner mitochondrial
membrane and thus plays a key role in steroid biosynthesis. To clarify the
implications of this protein in neurosteroid biosynthesis, we examined the
possible expression of a StAR transcript in the adult rat CNS and detected
it. cDNA cloning and sequencing analysis revealed that two forms of StAR
mRNAs are expressed in the brain in the same manner as in the adrenal
gland, indicating that they are fully functional and not minor gene
transcripts. An RNase protection assay quantitatively revealed that the
amount of the rat StAR transcript in brain was two to three orders of
magnitude lower than that in the adrenal gland. An in situ hybridization
study, involving antisense riboprobes, revealed that StAR transcripts
were abundant in the cerebral cortex, hippocampus, dentate gyrus, olfactory
bulb, cerebellar granular layer, and Purkinje cells. Furthermore, other
steroidogenic enzymes, side-chain cleavage cytochrome P-450SCC (CYP XIA1)
and 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase (EC
1.1.1.145), were found to be coexpressed in the hippocampus, dentate gyrus,
cerebellar granular layer, and Purkinje cells. These findings strongly
indicate that neurosteroids are synthesized in a region-specific manner in
the brain.


 10/3,AB/2     (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09805408   98238634   PMID: 9571148
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In vitro and in vivo potentiation of radiosensitivity of malignant gliomas by **antisense** inhibition of the RAD51 gene.

Ohnishi T; Taki T; Hiraga S; Arita N; Morita T
Department of Neurosurgery, Osaka University Medical School, Japan. ohnishi@nsurg.med.osaka-u.ac.jp

Biochemical and biophysical research communications (UNITED STATES) Apr 17 1998, 245 (2) p319-24, ISSN 0006-291X Journal Code: 0372516
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The mammalian RAD51 gene is a homologue of the yeast RAD51 and E. coli RecA genes, which are related to the repair of DNA double-strand breaks and are also involved in recombination repair and various SOS responses to DNA damage by gamma-irradiation and alkylating reagents. In this study, we investigated both in vitro and in vivo whether inhibition of the RAD51 gene by **antisense** oligonucleotides (ODNs) enhances the radiosensitivity of mouse malignant gliomas. A volume of 100 nM of RAD51 **antisense** ODNs inhibited the level of mRNA by more than 95% and reduced the protein expression by about 70%. Treatment of mouse 203G glioma cells with 100 nM of RAD51 **antisense** ODNs significantly enhanced the radiation-induced cell kill compared to control cells, and cells treated with sense or scrambled ODNs. When the glioma cells were implanted in the cisterna magna of mice followed by treatment with RAD51 **antisense** ODNs, the survival time of the mice was markedly prolonged compared to that of the untreated group ($p < 0.001$, logrank test). In addition, the combination of **antisense** ODNs and irradiation extended the survival time of the glioma-bearing mice much longer than could be achieved with radiation alone ($p < 0.0001$, logrank test). These results suggest that inhibition of RAD51 can be expected to serve as a novel potentiator for radiation therapy in malignant gliomas by inhibiting DNA double-strand break repair.

10/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08903269 96264658 PMID: 8670299

Antisense inhibition of the RAD51 enhances radiosensitivity.

Taki T; **Ohnishi T**; Yamamoto A; Hiraga S; Arita N; Izumoto S; Hayakawa T; Morita T
Department of Neurosurgery, Osaka University Medical School, Japan.

Biochemical and biophysical research communications (UNITED STATES) Jun 14 1996, 223 (2) p434-8, ISSN 0006-291X Journal Code: 0372516
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The mammalian RAD51 gene is a homologue of the yeast RAD51 and E. coli RecA genes, which are involved in recombination and DNA repair. We examined the role of RAD51 protein in mouse cells using RAD51 **antisense** phosphorothioate oligonucleotides (ODNs). The extraluminal delivery of 50 nM or 100 nM of **antisense** ODNs with lipofection to mouse cells resulted 90% suppression of RAD51 protein expression. The **antisense** ODNs significantly inhibited the cell growth and the treated cells became more sensitive to gamma-irradiation than the control groups. These results indicate mouse RAD51 plays an essential role in cell proliferation and radioresistant activity.

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PALM INTRANET

Day : Sunday
Date: 8/25/2002
Time: 16:15:16

Inventor Name Search Result

Your Search was:

Last Name = OHNISHI
First Name = TAKANORI

Application#	Patent#	Status	Date Filed	Title	Inventor Name
08477713	5596344	150	06/07/1995	DRIVING METHOD OF DRIVING A LIQUID CRYSTAL DISPLAY ELEMENT	OHNISHI , TAKANORI
08479348	5682177	150	06/07/1995	DRIVING METHOD OF DRIVING A LIQUID CRYSTAL DISPLAY ELEMENT	OHNISHI , TAKANORI
08083521	Not Issued	166	06/30/1993	DRIVING METHOD OF DRIVING A LIQUID CRYSTAL DISPLAY ELEMENT	OHNISHI , TAKANORI
08098812	5548302	150	07/29/1993	METHOD OF DRIVING DISPLAY ELEMENT AND ITS DRIVING DEVICE	OHNISHI , TAKANORI
08314435	5489910	150	09/28/1994	IMAGE DISPLAY DEVICE AND METHOD OF DRIVING THE SAME	OHNISHI , TAKANORI
07910513	5262881	150	07/08/1992	DRIVING METHOD OF DRIVING A LIQUID CRYSTAL DISPLAY ELEMENT	OHNISHI , TAKANORI
08133289	Not Issued	161	10/08/1993	DRIVING METHOD OF DRIVING A LIQUID CRYSTAL DISPLAY ELEMENT	OHNISHI , TAKANORI
60119506	Not Issued	159	02/10/1999	IN VITRO AND IN VIVO POTENTIATION OF RADIOSENSITIVITY OF MALIGNANT GLIOMAS BY ANTISENSE INHIBITION OF THE RAD51 GENE	OHNISHI , TAKANORI
08174262	5489919	150	12/28/1993	DRIVING METHOD OF DRIVING A LIQUID	OHNISHI , TAKANORI

				CRYSTAL DISPLAY ELEMENT	
09260624	Not Issued	071	03/01/1999	ANTISENSE INHIBITION OF RAD51	OHNISHI , TAKANORI
07973950	Not Issued	166	11/09/1992	IMAGE DISPLAY DEVICE AND A METHOD OF DRIVING THE SAME	OHNISHI , TAKANORI
08216948	Not Issued	161	03/24/1994	LIQUID CRYSTAL DISPLAY APPARATUS	OHNISHI , TAKANORI
08227342	Not Issued	166	04/14/1994	METHOD FOR FORMING COLUMN SIGNALS FOR A LIQUID CRYSTAL DISPLAY APPARATUS	OHNISHI , TAKANORI
08677912	5754157	150	07/10/1996	METHOD FOR FORMING COLUMN SIGNALS FOR A LIQUID CRYSTAL DISPLAY APPARATUS	OHNISHI , TAKANORI

Inventor Search Completed: No Records to Display.

Search Another: Inventor

Last Name OHNISHI **First Name** TAKANORI

Search

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WEST

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L1: Entry 52 of 65 File: USPT Jul 14, 1998

DOCUMENT-IDENTIFIER: US 5780296 A
TITLE: Compositions and methods to promote homologous recombination in eukaryotic cells and organisms

Detailed Description Text (12):
Alternative methods to isolate putative REC2 genes from other species of eukaryotes utilize the paired sense and antisense oligonucleotides, the sequences of which encode, or are complementary to nucleic acids encoding, the portions of Rec2 that are highly conserved among species. One such portion consists of residues 226-270, which shows homology with S. cerevisiae proteins Dmc1, Rad57 and Rad51 and with the E. coli protein RecA. The oligonucleotides are selected to bracket portions of the gene of about 100 to 500 bp. The paired oligonucleotides can be used as primers in a polymerase chain reaction (PCR) to amplify the bracketed fragment of the gene. The amplification products may then be cloned, sequenced and those, the sequence of which indicates that they are fragments of a Rec2 gene, can be used as probes to isolate the entire gene from a suitable library.

WEST

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L1: Entry 47 of 65 File: USPT Aug 31, 1999

DOCUMENT-IDENTIFIER: US 5945339 A
TITLE: Methods to promote homologous recombination in eukaryotic cells and organisms

Detailed Description Text (12):
Alternative methods to isolate putative REC2 genes from other species of eukaryotes utilize the paired sense and antisense oligonucleotides, the sequences of which encode, or are complementary to nucleic acids encoding, the portions of Rec2 that are highly conserved among species. One such portion consists of residues 226-270, which shows homology with S. cerevisiae proteins Dmc1, Rad57 and Rad51 and with the E. coli protein RecA. The oligonucleotides are selected to bracket portions of the gene of about 100 to 500 bp. The paired oligonucleotides can be used as primers in a polymerase chain reaction (PCR) to amplify the bracketed fragment of the gene. The amplification products may then be cloned, sequenced and those, the sequence of which indicates that they are fragments of a Rec2 gene, can be used as probes to isolate the entire gene from a suitable library.

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L1: Entry 39 of 65 File: USPT Feb 22, 2000

DOCUMENT-IDENTIFIER: US 6027892 A
TITLE: Compositions and methods for reducing radiation and drug resistance in cells

Brief Summary Text (13):
It is a further object of the present invention to provide a method to resensitize radiation- and drug-resistant cells, the method comprising administering to the cells antisense oligonucleotides of genes identified in the signal transduction pathway leading to resistance such as oligonucleotides of the present invention. Other genes involved in the MAPK signal transduction pathway are defined in FIG. 6. Applicants have found that growth factor sis (PDGF-.beta.), receptor tyrosine kinases trk (nerve growth factor), met (hepatocyte growth factor), tyrosine kinase src, serine/threonine kinase mos, protein kinase C .beta.-1, nuclear oncogene ets-1, as well as some other components of the MAPK pathway, are involved in the radiation resistant phenotype, and administration of oligonucleotides which block the transduction pathway through these and other genes in this pathway may reduce the radiation resistance phenotype [Pirollo, K. F. et al. (1993) Rad. Res. 135:234-243]. This method is important in the treatment of tumors, especially tumors which have acquired resistance to radiation and drugs, both endocrine and chemical.

Detailed Description Text (45):
These studies, supporting a pathway, with raf-1 as a central element, leading to cellular radioresistance are also clinically significant in a number of ways. Radiation is one of the major forms of adjuvant therapy for various types of cancer. Understanding the molecular mechanisms leading to the failure of a significant fraction of tumors to respond to radiotherapy opens the door to the development of new methods of intervention to radio-sensitize tumors, resulting in more effective cancer treatments. In this vein, our use of antisense oligonucleotides to radiosensitize human tumor cells not only establishes the signal transduction pathway, but also demonstrates the potential of these molecules as cancer therapeutic agents showing that ASO directed against a focal point in the pathway can be effective in a number of different tumor types. In a similar way, using mouse m5S cells, Taki et al. [Taki, T. et al. (1996) Bio. Biop. R. 223:834-438] also recently found that ASO against RAD51, a gene involved in recombination and DNA repair, could increase radiosensitivity.

L1: Entry 37 of 65 File: USPT Mar 14, 2000

DOCUMENT-IDENTIFIER: US 6037125 A
TITLE: Disruption of the mammalian RAD51 protein and disruption of proteins that associate with mammalian RAD51 for hindering cell proliferation and/or viability of proliferating cells

Detailed Description Text (15):
Typical examples of therapeutic agents based on the above presently described molecules include, but are not limited to, defective (either engineered or naturally occurring) forms of the proteins that associate with the protein complexes, inhibitory fragments of the proteins, wild type and altered genes that code for proteins that disrupt mammalian Rad51 function, small organic molecules, antisense nucleic acid sequences, oligonucleotides that inhibit expression or activity via a triplex mechanism, peptides, aptameric oligonucleotides, and the like.

Detailed Description Text (32):
Additionally, an alternative means for employing the presently disclosed anti-proliferation agents includes the use of vectors to directly insert genes encoding the agents into target cells (e.g., gene therapy). For example, when the tumor cells express the genes encoding the desired sequences, DSB repair will be disrupted and the tumor cell will die. Alternatively, one could attack tumor cells using a strategy conceptually similar to that disclosed in U.S. Pat. No. 5,529,774 herein incorporated by reference. In brief, cells that produce transducing virus encoding sequence that disrupts DSB repair may be implanted at or near the tumor mass. As the producer cells continue to elaborate virus, the growing tumor cells are infected and effectively killed as they expressing the agent that blocks DSB repair. The above methodology has proven useful in the treatment of glioblastomas and other tumors of the brain by using retroviral vectors to selectively target actively replicating tumor cells. A similar methodology could be used to deliver antisense sequences that target (and thus inhibit) the expression of Rad51 or any of the proteins involved in the Rad51 or Rad52 pathways.

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L1: Entry 35 of 65 File: USPT May 2, 2000

DOCUMENT-IDENTIFIER: US 6057104 A
TITLE: Disruption of the mammalian Rad51 protein and disruption of proteins that associate with mammalian Rad51 for hindering cell proliferation

Detailed Description Text (18):
Typical examples of therapeutic agents based on the above presently described molecules include, but are not limited to, defective (either engineered or naturally occurring) forms of the proteins that associate with the protein complexes, inhibitory fragments of the proteins, wild type and altered genes that code for proteins that disrupt mammalian Rad51 function, small organic molecules, antisense nucleic acid sequences, oligonucleotides that inhibit expression or activity via a triplex mechanism, peptides, aptameric oligonucleotides, and the like.

Detailed Description Text (65):
Additionally, an alternative means for employing the presently disclosed anti-proliferation agents includes the use of vectors to directly insert genes encoding the agents into target cells (e.g., gene therapy). For example, when the tumor cells express the genes encoding the desired sequences, DSB repair will be disrupted and the tumor cell will die. Alternatively, one could attack tumor cells using a strategy conceptually similar to that disclosed in U.S. Pat. No. 5,529,774 herein incorporated by reference. In brief, cells that produce transducing virus encoding sequence that disrupts DSB repair may be implanted at or near the tumor mass. As the producer cells continue to elaborate virus, the growing tumor cells are infected and effectively killed as they express the agent that blocks DSB repair. The above methodology has proven useful in the treatment of glioblastomas and other tumors of the brain by using retroviral vectors to selectively target actively replicating tumor cells. A similar methodology could be used to deliver antisense sequences that target (and thus inhibit) the expression of Rad51 or any of the proteins involved in the Rad51 or Rad52 pathways.

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L1: Entry 20 of 65 File: PGPB Nov 1, 2001

DOCUMENT-IDENTIFIER: US 20010036929 A1
TITLE: Xrcc3 is required for assembly of Rad51-complexes in vivo

Summary of Invention Paragraph (31):
[0029] The present invention also proposes methods for treating an animal or human patient with cancer. These methods generally comprise contacting cancer cells in the animal or human patient with a pharmaceutically acceptable composition comprising Rad51 antisense RNA or Xrcc3 antisense RNA. The invention also contemplates contacting the cancer cells of the patient with a DNA damaging agent. The pharmaceutically acceptable composition may be provided to the patient as a first polynucleotide encoding a Rad51 antisense RNA or a second polynucleotide encoding a Xrcc3 antisense RNA. A polynucleotide may be delivered to the animal patient as naked DNA or through viral delivery.

Summary of Invention Paragraph (32):
[0030] In a further aspect of the invention, the polynucleotides are under the control of a promoter operatively linked to the first and second polynucleotides, respectively. In one embodiment, the promoter is a radiation-inducible promoter. In another embodiment, the polynucleotides have a polyadenylation signal positioned 3' to the first and second polynucleotides, respectively. Rad51 antisense RNA or Xrcc3 antisense RNA also may be provided to the animal patient under the control of a selectable marker. In yet another embodiment, the polynucleotide is contained in a viral vector.

Summary of Invention Paragraph (33):
[0031] The invention also contemplates that both Rad51 antisense RNA and Xrcc3 antisense RNA are provided to said animal or human patient as polynucleotides encoding a Rad51 antisense RNA and a Xrcc3 antisense RNA, respectively. The polynucleotides may be delivered to the animal patient as naked DNA or through viral delivery. In yet another embodiment, the polynucleotides are contained in viral vectors. Rad51 antisense RNA and Xrcc3 antisense RNA may also be provided to the animal patient under the control of a selectable marker.

Detail Description Paragraph (7):
[0044] In another embodiment of the invention, Rad51, Xrcc3, or both Rad51 and Xrcc3, are administered to an animal patient to prevent or treat cellular damage that results from exposure to a DNA damaging agent. In an alternative embodiment of the present invention, Rad51 antisense RNA, Xrcc3 antisense RNA, or both antisense RNAs, are contacted with cancer cells in an animal patient to prevent the formation of the Rad51/Xrcc3 complex in the diseased cells. In a further embodiment, the cancer cells are then exposed to a DNA damaging agent. Since the cells will not have a functional Rad51/Xrcc3 complex to regulate DNA repair, they will be more susceptible to DNA damage and cell death. Included in the invention are treatments that involve cancers such as brain, lung, liver, spleen, kidney, lymph node, small intestine, pancreas, blood cells, colon, stomach, breast, endometrium, prostate, testicle, ovary, skin, head and neck, esophagus, bone marrow and blood tumors. The invention also is intended for use in benign neoplasms, including meningiomas, arteriovenous malformations, hemangiomas and the like. Cellular DNA damage may be caused by radiation or chemotherapeutic agents.

Detail Description Paragraph (24):
[0061] If Rad51 and Xrcc3 interact as a complex to facilitate DNA repair as the

inventors propose, a useful strategy to increase the susceptibility of certain cells, such as cancerous cells, to DNA damaging agents may be to prevent the formation of, or block the expression of, the Rad51/Xrcc3 complex. While Rad51 is essential for viability, Xrcc3 is not essential for the survival of eukaryotic cells. Antisense RNA treatments are one way of blocking the expression of Xrcc3 in a cell. Antisense technology also may be used to "knock-out" the function of Xrcc3 in the development of cell lines or transgenic mice for research, diagnostic and screening purposes, or therapeutic purposes. In addition, Rad51 function in a cell may also be reduced by antisense RNA treatments, potentially increasing the sensitivity of a cell to DNA damaging agents.

Detail Description Paragraph (168):
[0200] The present invention includes, in another embodiment, the treatment of cancer through the functional inactivation of the Rad51/Xrcc3 complex. The types of cancer that may be treated are limited only by the involvement of Rad51 or Xrcc3. By involvement, it is not a requirement that either Rad51 or Xrcc3 be mutated or abnormal. Since the formation of a Rad51/Xrcc3 complex appears to regulate DNA damage repair, it is contemplated that preventing the formation of the complex in a cell using an antisense RNA strategy will enhance the susceptibility of a cell to DNA damaging agents. A single-chain antibody strategy may also be used to block the function of the Rad51/Xrcc3 complex in a cell. A wide variety of cancers may be treated using such a strategy, including cancers of the brain (glioblastoma, astrocytoma, oligodendroglioma, ependymomas), lung, liver, spleen, kidney, lymph node, pancreas, small intestine, blood cells, colon, stomach, breast, endometrium, prostate, testicle, ovary, skin, head and neck, esophagus, bone marrow, blood or other tissue. The invention may also be used in benign neoplasms, including meningiomas, arteriovenous malformations, and hemangiomas.

Detail Description Paragraph (171):
[0203] One therapeutic embodiment contemplated by the present invention is the intervention, at the molecular level, in the events involved in cellular DNA repair. In particular, the present inventors intend to prevent the formation of the Rad51/Xrcc3 complex in cancer cells within an animal patient. The efficacy of radiotherapy and chemotherapy will be improved if a strategy can be used to specifically increase the susceptibility of cancer cells to DNA damaging agents in a patient. Formation of the Rad51/Xrcc3 complex can be blocked by expressing Rad51 antisense RNA, Xrcc3 antisense RNA, or both antisense RNAs in cancer cells. The inventors propose that the absence of the Rad51/Xrcc3 complex will enhance the susceptibility of cancer cells to DNA damaging agents because the Rad51/Xrcc3 complex will not be present to aid in DNA repair. The lengthy discussion of polynucleotides employed herein is incorporated into this section by reference. Particularly preferred polynucleotides are contained in viral vectors such as adenovirus, adeno-associated virus, herpesvirus, vaccinia virus and retrovirus.

Detail Description Paragraph (172):
[0204] Various routes are contemplated for delivery of Rad51 antisense RNA, Xrcc3 antisense RNA, or both antisense RNAs to cancer cells in an animal patient. The discussion on routes for administration to patients employed herein is incorporated into this section by reference. Systemic delivery in animal patients is contemplated by the invention. If a discrete tumor mass is identified in a patient, a variety of direct, local and regional approaches may be taken.

Detail Description Paragraph (174):
[0206] Tumor cell resistance to DNA damaging agents represents a major problem in clinical oncology. One goal of current cancer research is to find ways to improve the efficacy of chemotherapy and radiotherapy. One way is by combining such traditional therapies with gene therapy. For example, the herpes simplex-thymidine kinase (HS-tk) gene, when delivered to brain tumors by a retroviral vector system, successfully induced susceptibility to the antiviral agent ganciclovir (Culver et al., 1992). In the context of the present invention, it is contemplated that Rad51 antisense RNA, Xrcc3 antisense RNA, or both antisense RNAs could similarly be used in conjunction with chemotherapeutic or radiotherapeutic intervention.

Detail Description Paragraph (175):
[0207] To kill cells, inhibit cell growth, inhibit metastasis, inhibit angiogenesis

or otherwise reverse or reduce the malignant phenotype of tumor cells using the methods and compositions of the present invention, one would generally contact a "target" cell with Rad51 antisense RNA, Xrcc3 antisense RNA, or both antisense RNA expression constructs and at least one other DNA damaging agent. These compositions would be provided in a combined amount effective to kill or inhibit proliferation of the cell. This process may involve contacting the cells with the antisense RNA expression constructs and the agent(s) or factor(s) at the same time. This may be achieved by contacting the cell with a single composition or pharmacological formulation that includes both agents, or by contacting the cell with two distinct compositions or formulations, at the same time, wherein one composition includes the expression constructs and the other includes the agent.

Detail Description Paragraph (176):
[0208] Alternatively, the Rad51 antisense RNA, Xrcc3 antisense RNA, or both antisense RNA treatments may precede or follow the other DNA damaging agent treatment by intervals ranging from minutes to weeks. In embodiments where the other agent and antisense RNA expression constructs are applied separately to the cell, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the agent and expression constructs would still be able to exert an advantageously combined effect on the cell. In such instances, it is contemplated that one would contact the cell with both modalities within about 12-24 hours of each other and, more preferably, within about 6-12 hours of each other, with a delay time of only about 12 hours being most preferred. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

Detail Description Paragraph (177):
[0209] It is also conceivable that more than one administration of either Rad51 antisense RNA, Xrcc3 antisense RNA, both antisense RNAs, or the other agent will be desired. Various combinations may be employed, where Rad51 antisense RNA, Xrcc3 antisense RNA, or both antisense RNAs are "A" and the other DNA damaging agent is "B", as exemplified below:

Detail Description Paragraph (179):
[0211] Agents or factors suitable for use in a combined therapy are any chemical compound or treatment method that induces DNA damage when applied to a cell. Such agents and factors include radiation and waves that induce DNA damage, such as .gamma.-irradiation, X-rays, UV-irradiation, microwaves, electronic emissions, and the like. A variety of chemical compounds, also described as "chemotherapeutic agents," function to induce DNA damage, all of which are intended to be of use in the combined treatment methods disclosed herein. Chemotherapeutic agents contemplated to be of use, include, e.g., adriamycin, 5-fluorouracil (5FU), etoposide (VP-16), camptothecin, actinomycin-D, mitomycin C, cisplatin (CDDP) and even hydrogen peroxide. The invention also encompasses the use of a combination of one or more DNA damaging agents, whether radiation-based or actual compounds, such as the use of X-rays with cisplatin or the use of cisplatin with etoposide. In certain embodiments, the use of cisplatin in combination with Rad51 antisense RNA, Xrcc3 antisense RNA, or both antisense RNA expression constructs is particularly preferred as this compound.

Detail Description Paragraph (180):
[0212] In treating cancer according to the invention, one would contact the tumor cells with a DNA damaging agent in addition to antisense RNA expression constructs. This may be achieved by irradiating the localized tumor site with radiation such as X-rays, UV-light, .gamma.-rays or even microwaves. Alternatively, the tumor cells may be contacted with the agent by administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a compound such as adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin-D, mitomycin C, or more preferably, cisplatin. The agent may be prepared and used as a combined therapeutic composition, or kit, by combining it with Rad51 antisense RNA, Xrcc3 antisense RNA, or both antisense RNA expression constructs, as described above.

Detail Description Paragraph (182):
[0214] The inventors propose that the regional delivery of Rad51 antisense RNA,

Xrcc3 antisense RNA, or both antisense RNA expression constructs to patients with cancer will be a very efficient method for counteracting the clinical disease. Similarly, the chemotherapy or radiotherapy may be directed to a particular, affected region of the subjects body. Alternatively, systemic delivery of antisense RNA expression constructs and/or the DNA damaging agent may be appropriate in certain circumstances, for example, where extensive metastasis has occurred.

CLAIMS:

53. A method for treating an animal patient with cancer comprising contacting cancer cells in said patient with a pharmaceutically acceptable composition comprising Rad51 antisense RNA or Xrcc3 antisense RNA, wherein said antisense RNA blocks the formation of a functional Rad51/Xrcc3 complex.

55. The method of claim 53, wherein said Rad51 antisense RNA or said Xrcc3 antisense RNA is provided to said animal patient as a first polynucleotide encoding a Rad51 antisense RNA or a second polynucleotide encoding a Xrcc3 antisense RNA.

60. The method of claim 55, wherein said Rad51 antisense RNA or said Xrcc3 anti sense RNA is provided to said animal patient under the control of a selectable marker.

62. The method of claim 36, wherein said Rad51 antisense RNA and said Xrcc3 antisense RNA are both provided to said animal patient as polynucleotides encoding a Rad51 antisense RNA and a Xrcc3 antisense RNA, respectively.

64. The method of claim 62, wherein both said Rad51 antisense RNA and said Xrcc3 antisense RNA are provided to said animal patient under the control of a selectable marker.

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L1: Entry 6 of 65 File: PGPB Jul 4, 2002

DOCUMENT-IDENTIFIER: US 20020086840 A1
TITLE: Use of Rad51 inhibitors for p53 gene therapy

Abstract Paragraph (1):
The present invention is directed to methods and compositions for inhibiting or reducing tumor cell proliferation in an individual in vivo. More specifically, a tumor cell is contacted, in vivo, with a Rad51 inhibitor, and a polynucleotide capable of expressing functional p53 protein. In a further embodiment of the present invention the tumor cell is exposed in vivo to radiation or chemotherapeutic agents (e.g., BCNU, CCNU, and DMZ, GB, cisplatin and the like). The Rad51 inhibitor may be selected from the group consisting of peptides, small molecules and Rad51 antisense molecules. The Rad51 antisense molecule and the p53 polynucleotide may be encoded on an expression vector under the control of one or more promoters, and the expression vector may then be incorporated into a viral genome, preferably an andeno or retro virus, which is then used to introduce the expression vector into the tumor cell.

Summary of Invention Paragraph (20):
[0017] The present invention is directed to methods and compositions for inhibiting or reducing tumor cell proliferation in an individual in vivo. More specifically, a tumor cell is contacted, in vivo, with a Rad51 inhibitor, and a polynucleotide capable of expressing functional p53 protein. In a further embodiment of the present invention the tumor cell is exposed in vivo to radiation or chemotherapeutic agents (e.g., BCNU, CCNU, and DMZ, GB, cisplatin and the like). The Rad51 inhibitor may be selected from the group consisting of peptides, small molecules and Rad51 antisense molecules. The Rad51 antisense molecule and the p53 polynucleotide may be encoded on an expression vector under the control of one or more promoters, and the expression vector may then be incorporated into a viral genome, preferably an andeno or retro virus, which is then used to introduce the expression vector into the tumor cell.

Detail Description Paragraph (9):
[0025] Rad51 inhibitors are preferably selected from the group consisting of small molecules, Rad51 antisense molecules, and pepetides.

Detail Description Paragraph (17):
[0033] In a preferred embodiment, the Rad51 inhibitors are nucleic acids. By "nucleic acid" or "oligonucleotide" or grammatical equivalents herein means at least two nucleotides covalently linked together. A nucleic acid of the present invention will generally contain phosphodiester bonds. However, in some cases, as outlined below, nucleic acid analogues are included that may have alternate backbones, comprising, for example, phosphoramide (Beaucage et al., Tetrahedron 49(10):1925 (1993); Letsinger, J. Org. Chem. 35:3800 (1970); Sprinzl et al., Eur. J. Biochem. 81:579 (1977); Letsinger et al., Nucl. Acids Res. 14:3487 (1986); Sawai et al, Chem. Lett. 805 (1984); Letsinger et al., J. Am. Chem. Soc. 110:4470 (1988); Pauwels et al., Chemica Scripta 26:141 (1986)), phosphorothioate (Mag et al., Nucleic Acids Res. 19:1437 (1991); U.S. Pat. No. 5,644,048), phosphorodithioate (Briu et al., J. Am. Chem. Soc. 111:2321 (1989)), O-methylphosphoroamidite linkages (Eckstein, Oligonucleotides and Analogues: a Practical Approach (Oxford University Press)), and peptide nucleic acid backbones and linkages (Egholm, J. Am. Chem. Soc. 114:1895 (1992); Meier et al., Chem. Int. Ed. Engl. 31:1008 (1992); Nielsen, Nature 365:566 (1993); Carlsson et al., Nature 380:207 (1996)). Other analogue nucleic acids include those with positive backbones (Denpcy et al., Proc. Natl. Acad. Sci. USA 92:6097 (1995)), non-ionic backbones (U.S. Pat. Nos. 5,386,023, 5,637,684,

5,602,240, 5,216,141, 4,469,863; Kiedrowshi et al., Angew. Chem. Intl. Ed. English 30:423 (1991); Letsinger et al., J. Am. Chem. Soc. 110:4470 (1988); Letsinger et al., Nucleoside & Nucleotide 13:1597 (1994); Chapters 2 and 3, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y. S. Sanghui and P. Dan Cook; Mesmaeker et al., Bioorganic & Medicinal Chem. Lett. 4:395 (1994); Jeffs et al., J. Biomolecular NMR 34:17 (1994); Tetrahedron Lett. 37:743 (1996)), and non-ribose backbones (U.S. Pat. Nos. 5,235,033, 5,034,506; Chapters 6 and 7, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y. S. Sanghui and P. Dan Cook). Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids (Jenkins et al., Chem. Soc. Rev. pp169-176 (1995)). Several nucleic acid analogues are described in Rawls, C & E News p.35 (Jun. 2, 1997). All of these references are incorporated herein, in their entirety, by reference. These modifications of the ribose-phosphate backbone may be done to facilitate the addition of additional moieties such as labels, or to increase the stability and half-life of such molecules in physiological environments. In addition, mixtures of naturally occurring nucleic acids and analogs including PNA can be made. Alternatively, mixtures of different nucleic acid analogs, and mixtures of naturally occurring nucleic acids and analogs may be made. The nucleic acids may be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA or a hybrid (where the nucleic acid contains any combination of deoxyribo- and ribo-nucleotides). The nucleic acid may contain any combination of bases, including without limitation uracil, adenine, thymine, cytosine, guanine, inosine, xathanine hypoxathanine, isocytosine, and isoguanine.

Detail Description Paragraph (20):
[0036] In an alternative embodiment the nucleic acid Rad51 inhibitor is a Rad51 antisense molecule. Preferably the Rad51 antisense molecule is at least about 10 nucleotides in length, more preferably at least 12, and most preferably at least 15 nucleotides in length. In an alternative embodiment the Rad51 antisense molecule is a morpholino based antisense molecule. Nasevicius, A. and Eker, S., Nature Genetics, 26(2):216-220 (2000); Heasman et al. Developmental Biology, 222:124-134 (2000). The skilled artisan understands that the length can extend from 10 nucleotides or more to any length which still allows binding to the Rad51 mRNA. Preferably, the length is about 30 nucleotides, more preferably about 25 nucleotides, and most preferably about 12 to 25 nucleotides in length.

Detail Description Paragraph (21):
[0037] The Rad51 antisense molecules hybridize under normal intracellular conditions to the target nucleic acid to inhibit Rad51 expression or translation. In an alternative embodiment an anti-gene may be used. The target nucleic acid is either DNA or RNA. In one embodiment, the antisense molecules bind to regulatory sequences for Rad51. Alternatively, the antisense molecules bind to 5' or 3' untranslated regions directly adjacent to the coding region of the Rad51 gene. Preferably, the antisense molecules bind to the nucleic acid within 1000 nucleotides of the coding region, either upstream from the start or downstream from the stop codon. In a preferred embodiment, the antisense molecules bind within the coding region of the Rad51 gene. More preferably, the Rad51 antisense molecule is selected from the group consisting of AS4, AS5, AS6, AS7, AS8 and AS9 as indicated in FIG. 1 and Table 1 below. Table 1 includes the recitation of "R51" before the same corresponding antisense, but "AS4" and "R51AS4", for example, are used interchangeably herein. In one embodiment, the Rad51 antisense molecules are not directed to the structural gene; this embodiment is particularly preferred when the Rad51 antisense molecule is not combined with another antisense molecule. It is understood that any of the antisense molecules can be combined.

Detail Description Paragraph (24):
[0040] In a preferred embodiment of the present invention, the expression vector has a first polynucleotide that encodes functional p53 protein, a second polynucleotide encoding a Rad51 antisense molecule, a first promoter for the p53 polynucleotide, a second promoter for the Rad51 antisense molecule, and a polyadenylation signal. In one embodiment the first and second promoters may be the same. The expression vector is then incorporated into a replication-deficient adenovirus, or other suitable transfection vehicle, and introduced into a diseased cell. The infection results in

the expression of functional p53 protein in the diseased cells and the transcription of Rad51 antisense molecule. The combination of the functional p53 protein and the inhibition of Rad51 activity results in the reduction and inhibition of diseased cell proliferation.

Detail Description Paragraph (25):
[0041] Alternatively, the Rad51 antisense (or other Rad51 inhibitor as discussed herein), and functional p53 protein are delivered to a diseased cell, thereby eliminating the need for introducing an expression vector to the diseased cell, as described above. As will be appreciated by the skilled artisan, any combination of the the techniques for delivering Rad51 inhibitor, and functional p53 protein to a diseased cell may be used.

Detail Description Paragraph (26):
[0042] As described above and in addition to Rad51 antisense molecules, the Rad51 inhibitor used in combination with p53 gene therapy may be selected from the group consisting of small molecules (including nucleotides and analogues thereof, as described above), or peptides

CLAIMS:

3. The method according to claim 1 or 2, wherein said Rad51 inhibitor is selected from the group consisting of Rad51 antisense molecules, small molecules, peptides or antibodies.

4. The method according to claim 1 or 2, wherein said Rad51 inhibitor is a Rad51 antisense molecule.

5. The method according to claim 4, wherein the step of contacting said antisense molecule further comprises: introducing to said tumor cell in vivo an expression vector comprising a eukaryotic functional promoter and a polynucleotide sequence encoding a Rad51 antisense molecule, wherein said polynucleotide sequence is under transcriptional control of said eukaryotic functional promoter.

8. The method according to claim 4 further comprising introducing to said tumor cell in vivo an expression vector comprising: (i) a first polynucleotide sequence encoding a Rad51 antisense molecule; and (ii) a second polynucleotide sequence encoding said functional p53 protein, wherein said first and second polynucleotides are operably linked to one or more promoter sequences which are functional in said tumor cell to produce said Rad51 antisense molecule and said functional p53 protein

9. The method according to claim 4, wherein said Rad51 antisense molecule is selected from the group consisting of AS4, AS5, AS6, AS7, AS8 and AS9.

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L2: Entry 1 of 4 File: USPT May 2, 2000

US-PAT-NO: 6057104
DOCUMENT-IDENTIFIER: US 6057104 A

TITLE: Disruption of the mammalian Rad51 protein and disruption of proteins that associate with mammalian Rad51 for hindering cell proliferation

DATE-ISSUED: May 2, 2000

INVENTOR-INFORMATION:

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Lexicon Genetics Incorporated	The Woodlands	TX			02

APPL-NO: 08/ 964614 [PALM]
DATE FILED: November 5, 1997

PARENT-CASE:
The present application is a continuation-in-part of and claims priority to U.S. applications Ser. Nos. 08/758,280, filed Nov. 5, 1996. The disclosure of the above application is herein incorporated by reference.

INT-CL: [07] C12 Q 1/68

US-CL-ISSUED: 435/6; 435/196, 530/350, 536/23.2, 536/23.5
US-CL-CURRENT: 435/6; 435/196, 530/350, 536/23.2, 536/23.5

FIELD-OF-SEARCH: 435/6, 435/196, 530/350, 536/23.2, 536/23.5

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
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ART-UNIT: 166

PRIMARY-EXAMINER: Ketter; James

ABSTRACT:

When a mutation, designated rad51.sup.M1, was generated in the mouse MmRAD51 gene, mutant embryos died shortly after implantation. rad51.sup.M1 cells exhibited hypersensitivity to ionizing radiation, reduced proliferation, programmed cell death and chromosome loss. The disruption of MmRad51 protein--protein interactions stopped cell proliferation and/or reduced cell viability. Several proteins that interact with MmRad51 have been identified including, for example Brca2 and M96. Additionally, Rad51 self-associates via the N-terminal region. When a single residue was changed from a conserved lysine to an alanine, the alteration proved toxic to cells. Moreover, a rad51 allele that lacked the RecA homology region was also deleterious to cells. In view of the above, it is clear that inhibiting MmRad51 function or the function of any molecule that associates with MmRad51, or any molecule in the Rad51 or Rad52 pathways, hinders cell proliferation and/or viability. Accordingly, molecules capable of blocking these critical DNA repair pathways may be effective as therapeutics for inhibiting cell proliferation.

12 Claims, 5 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 3

BRIEF SUMMARY:

1 1.0. FIELD OF THE INVENTION

2 The present invention relates to molecules that disrupt mammalian Rad51 or Rad52 function, or disrupt the function of other molecules that are involved in the Rad51 or Rad52 pathways. Such molecules are useful as a means to hinder cell proliferation or to promote programmed cell death, and define a novel class of therapeutic agents for use in the treatment of proliferative disorders such as autoimmune disease and cancer.

3 2.0. BACKGROUND OF THE INVENTION

4 DNA repair and recombination are required by organisms to prevent the accumulation of mutations and to maintain the integrity of genetic information. Compromised genetic material may result in cell cycle arrest, programmed cell death, chromosome loss or cell senescence. Alternatively, compromised genetic information may result in dysregulation of the cell cycle ultimately leading to increased cellular growth and tumor formation.

5 The repair of double-strand breaks (DSB) in DNA is an essential cellular process. DSB repair may occur during general cellular functions such as DNA repair (Friedberg et al., 1995, DNA Repair and Mutagenesis. American Society for Microbiology, Washington, D.C.). In bacteria and yeast cells, DSB are predominately repaired by a homologous recombination pathway (Krasin and Hutchinson, 1977, J. Mol. Biol. 116:81-98; Mortimer, 1958, Radiat. Res. 9:312-16. In the budding yeast Saccharomyces cerevisiae the RAD52 epistasis group (Rad.sub.50 to Rad57, Mre11 and Xrs2) was identified in cells sensitive to ionizing radiation (reviewed in Friedberg, 1995; Petes et al., 1991, Recombination in yeast., p. 407-521. In J. R. P. J. R. Broach, and E. W. Jones (ed.), The Molecular and Cellular Biology of the Yeast Saccharomyces. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). Later, some of the members of this group were shown to be important for recombinational repair (e.g., Rad51, Rad52, Rad54, Rad55, Rad57 (Malkova et al., 1996, Proc. Natl. Acad. Sci. USA 93:7131-36, Sugawara et al., 1995, Nature 373:84-86).

6 Among the members of the RAD52 epistasis group, ScRad51 is particularly interesting because it shares similarity with the Escherichia coli recombination protein, RecA. ScRad51 and RecA polymerize on double-stranded and

single-stranded DNA (dsDNA, ssDNA) to produce a helical filament, and both enzymes catalyze an ATP-dependent strand exchange between homologous DNA molecules (Ogawa et al., 1993, Science 259:1896-99; Sung, 1994, Science 265:1241-4364; Sung and Robberson, 1995, Cell 82:453-61). ScRad51 and RecA share 30% homology over a span of about 220 amino acids, and each protein contains two conserved ATP binding motifs (Aboussekhra et al., 1992, Mol. and Cell. Biol. 12:3224-34; Basile et al., 1992, Mol. Cell. Biol. 12:3235-46; Sugawara et al., 1995, Nature 373:84-86).

7 ScRad51 repairs DSB by homologous recombination. DSB accumulate at recombination hot spots during meiosis in cells that lack ScRad51 (Sugawara, 1995), and ScRad51 localizes to meiotic nuclei (Bishop, 1994, Cell 79:1081-92) and promotes meiotic chromosome synapsis (Rockmill et al., 1995, Genes & Develop. 9:2684-95). Accordingly, it is thought that ScRad51 mediates meiotic recombination by binding to single-strands generated at DSB which are in strand pairing and exchange during meiosis (Sung and Robberson, 1995, Cell 82:453-61).

8 Direct and indirect protein--protein interactions are essential for RecA and ScRad51 function. The crystal structure of RecA suggests that a portion of the N-terminal region is involved in polymer formation (Story et al., 1993, Science 259:1892-96; Story et al., 1992, Nature 355:318-324) which was supported by genetic analysis that showed C-terminal truncations dominantly interfered with DNA repair in wild-type bacteria (Horii et al., 1992, J. Mol. Biol. 223:104-114; Tateishi et al., 1992, J. Mol. Biol. 223:115-129; Yarranton et al., 1982, Mol. Gen. Genet. 185:99-104). A similar self-association region occurs in the N-terminal region of ScRad51 and is essential for DNA repair (Donovan et al., 1994, Genes & Develop. 8:2552-2562; Shinohara et al., 1992, Cell 69:457-70). ScRad51 also associates with Rad52 and Rad55 (Hays et al., 1995, Proc. Natl. Acad. Sci USA 92:6925-6929; Johnson and Symington, 1995, Molec. Cell. Biol. 15(9):4843-4850; Milne and Weaver, 1993, Genes & Develop. 7:1755-1765) as well as other proteins. Other protein interactions may be inferred because a rad51 rad52A strain of S. cerevisiae was only partially complemented by Rad51 and Rad52 from Kluyveromyeces lactis (Donovan et al., 1994, Genes & Develop. 8:2552-2562), and because ScRad51 colocalized with Dmc1 to the synaptonemal complex (Bishop, 1994, Cell 79:1081-92). These data suggest that a large protein complex is necessary for recombinational repair and that disruption of any of the proteins in this complex hinders the repair of DSB.

9 RecA/ScRad51 homologues have been discovered in a wide range of organisms including the fission yeast Schizosaccharomyces pombe (Jang et al., 1994, Gene 142:207-11; Muris et al., 1993, Nuc. Acids Res. 21:4586-91; Shinohara et al., 1993, Nature Genet. 4:239-4358), lilies (Terasawa et al., 1995, Genes & Develop. 9:925-34), chickens (Bezzubova et al., 1993, Nucl. Acids Res. 21:1577-80), mice (Morita et al., 1993, Proc. Natl. Acad. Sci USA 90:6577-80; Shinohara et al. 1993, Nature Genet. 4:239-43) and humans (Shinohara et al. 1993; Yoshimura et al., 1993, Nucl. Acids Res. 21:1665), and appear to be involved in DNA repair and recombination based on the following evidence: 1) Conserved RecA homology--MmRad51 is 83% homologous, 69% identical to ScRad51, and 51% homologous, 28% identical to RecA. Shared homology between mammalian and yeast Rad51 suggest conserved function due to the remarkable similarity between other mammalian and yeast DNA repair pathways (reviewed in Cleaver, 1994, Cell 76:1-4); 2) Expression pattern--MmRAD51 is highly expressed in tissues involved in meiotic recombination such as testes (Morita et al., 1993, Proc. Natl. Acad. Sci USA 90:6577-80) and ovaries (Shinohara et al., 1993, Nature

10 Genet. 4:239-43). Additionally, expression of the S. pombe MmRad51 homologue SpRAD51 increased after cells were treated with methyl methanesulfonate which provides further evidence of a DNA repair function (Jang et al., 1994, Gene 142:207-11); 3) Protein cellular localization--Mouse, chicken, and lily Rad51 localizes at discrete foci on meiotic chromosomes at varying concentrations during prophase 1, possibly on the lateral elements and recombination nodules, which suggests a role in the repair of DSB during meiotic recombination (Ashley et al., 1995, Chromosoma 104:19-28; Haaf et al., 1995, Proc. Natl. Acad. Sci. USA 92:2298-2302; Terasawa et al., 1995). Moreover, increasing concentrations

of human Rad51, HsRad51, localize to the nucleus after exposure to DNA damaging agents which also suggests a repair function (Terasawa et al., 1995); 4) Filament formation on DNA--HsRad51 bind to ssDNA which demonstrates a potential for strand exchange (Benson et al., 1994, EMBO 13:5764-71); 5) Mouse cells with a rad51 mutation, designated rad51.sup.M1, displayed features that are known to be characteristic of unrepaired DSB in yeast cells (Lim and Hasty, 1996, In press) which include reduced proliferation, hypersensitivity to .gamma.-radiation, chromosome loss and programmed cell death.

11 3.0. SUMMARY OF THE INVENTION

12 An object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting mammalian Rad51 function.

13 An additional object is to hinder cell proliferation or reduce cell viability by disrupting mammalian Rad52 function.

14 Another object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting proteins that associate with mammalian Rad51.

15 Another object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting proteins that associate with mammalian Rad52.

16 Another object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting any proteins involved in the mammalian Rad51 or mammalian Rad52 pathways.

17 Another object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting mammalian Rad.sub.51 protein interactions.

18 Another object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting mammalian Rad52 protein interactions.

19 Another object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting protein--protein interactions that are involved in the mammalian Rad51 or mammalian Rad52 pathways.

20 Yet another embodiment of the present invention involves methods of identifying compounds that are capable of inhibiting the binding or function of any protein involved in the Rad51 pathway, and, in particular, compounds capable of binding or inhibiting the function of Rad51 protein. Accordingly, an additional embodiment of the present invention involves methods of screening for compounds that disrupt double-stranded break repair by assaying for microsatellite formation in cells; assaying for chromosome loss in cells; assaying for the disruption of strand exchange in an in vitro assay; assaying for decreased cell proliferation; assaying for premature replicative cellular senescence; and assaying for increased cell death.

21 Another object of the invention is to identify compounds capable of interfering with protein--protein interactions involved in DSB repair by screening large numbers of compounds in assays that allow the detection of a decrease in protein--protein interactions. In a further object of the invention, structural analysis of proteins, peptides, and compounds useful for modulating DSB repair is used to improve the modulation of DSB repair by new or known proteins, peptides, and compounds.

22 An additional object of the present invention are compounds that hinder cell proliferation or reduce cell viability by disrupting mammalian Rad51 function.

23 An additional object of the present invention are compounds that hinder cell proliferation or reduce cell viability by disrupting mammalian Rad52 function.

DRAWING DESCRIPTION:

4.0. BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. mRNA structure of MmRAD51. The predicted amino acids are numbered according to Shinohara et al., 1993. The shaded box represents the recA homology region. The open boxes represent regions that are not conserved across species. The thick vertical lines represent the ATP binding domains.

FIG. 2. MmRad51 self-association as demonstrated by the yeast two-hybrid system. The self-association is restricted to the most N-terminal 43 amino acids. The shaded box is the RecA core homology region (Shinohara et al, 1993). The thick vertical lines represent the ATP-binding sites. The open boxes represent regions that are not conserved between species. The relative .beta.-galactosidase (.beta.-gal) activities are presented, right panel. Full length wild-type MmRad51 is considered to be 100%. E12 served as a negative control and had 1% relative activity.

FIG. 3. Targeting the transgenes to the hprt locus. The hprt sequences contain exons 2 and 3 (labeled boxes). Hprt homology of vector origin is a thick line, of chromosomal origin is a thin line. The bacterial plasmid is represented by a wavy line. Potential locations for crossovers are X's labeled 1 or 2. Two recombination events are possible: either gene replacement with crossovers at both 1 and 2 or vector insertion with crossovers at either 1 or 2. For vector insertion, only a crossover at 1 is shown.

FIGS. 4A and 4B. Disruption of mammalian Rad51 function in cells. A) Conditional expression of mammalian Rad51 1-43 in ES cells increases sensitivity to gamma-radiation. Transgene turned on without Dox and turned off with Dox. Ten clones were observed and the averages are presented. B) Brca2 peptide decreases proliferation of p53--/-- fibroblasts. 50 micromolar concentration used. Colony size is based on cell number. The average of two experiments is shown when 100 cells were plated onto a 6 cm plate. The control is either no peptide or the 16 amino acid peptide derived from Antennapedia.

DETAILED DESCRIPTION:

1 4.0. DETAILED DESCRIPTION OF THE INVENTION

2 As discussed above, one embodiment of the present invention is the expression of altered mammalian rad51 alleles that disrupt mammalian Rad51 function, mammalian Rad52 function, or the function of any other protein in the mammalian Rad51 or Rad52 pathways. The function of MmRad51 is not entirely known; however, it is likely that it has the same function as ScRad51 which is recombinational repair. The recombinational repair pathway appears to be at least partially conserved between yeast and mammals. Mammalian homologues were found for members of the Rad52 epistasis group (Rad51, Rad52) and to other yeast proteins (Dmc1) implicated in recombinational repair (Malkova et al., 1996, Proc. Natl. Acad. Sci. USA 93:7131-36; Resnick et al., 1989, Proc. Natl. Acad. Sci. USA 86:2276-80; Tsuzuki et al., 1996, Proc. Natl. Acad. Sci USA 93:6236-40). Expression pattern supported the hypothesis that these homologues maintained the same function from yeast to mammals. MmRAD51 was highly expressed in tissues with cells involved in meiotic recombination, testis and ovary, and rapid cell division, intestine, embryo, and thymus (Morita et al., 1993; Shinohara et al., 1993). A role during meiotic recombination was further suggested because MmRAD51 was highly enriched in the synaptonemal complex in pachytene spermatocytes (Ashley et al., 1995; Haaf et al., 1995).

3 The most compelling evidence that MmRad51 and ScRad51 function is conserved comes from analysis of rad51 mutant cells, the mutation was designated rad51.sup.M1 (Lim and Hasty, 1996). rad51.sup.M1 cells exhibited reduced proliferation, hypersensitivity to .gamma.-radiation, chromosome loss and cell death. These characteristics were similar to yeast cells deficient for recombinational repair either due to sequence divergence or due to a mutation

in rad51 or rad52. Even though these data suggest MmRad51 functions during recombinational repair it is possible that the severe phenotype in rad51.sup.M1 cells was due to disruption of another process.

4 There is evidence that the RecA homologues perform multiple tasks, some of them not shared by the others. Two RecA homologues were found in Myxococcus xanthus, only one was essential, but both complemented UV sensitivity in an E. coli recA strain (Norioka et al., 1995, J. Bacteriol. 177:4179-82). Two RecA homologues found in yeast, ScRad51 and Dmc1, are essential for meiotic recombination, but only ScRad51 is essential for mitotic recombination (Bishop, 1994, Rockmill et al. 1995, Genes & Develop. 9:2684-95). In mammals, a Dmc1 homologue was isolated suggesting that RecA homologues possess diverse and unique functions in mammalian cells (Habu et al., 1996).

5 Reduced proliferation, hypersensitivity to .gamma.-radiation, chromosome loss, and cell death have all been associated with rad51.sup.M1 cells. These characteristics are similar to those seen in yeast cells deficient for recombinational repair either due to sequence divergence, or due to a mutation in rad51 or rad52 (Malkova et al., 1996, Proc. Natl. Acad. Sci. USA 93:7131-36; Resnick et al., 1989, Proc. Natl. Acad. Sci. USA 86:2276-80; Tsuzuki et al., 1996, Proc. Natl. Acad. Sci USA 93:6236-40). Even though these data suggest MmRad51 functions during recombinational repair it is also possible that the severe phenotype observed in rad51.sup.M1 cells was due to disruption of another process.

6 For the purposes of the present application the term ionizing radiation shall mean all forms of radiation, including but not limited to a, P, and T radiation and U.V. light, which are capable of directly or indirectly damaging the genetic material of a cell or virus. The term irradiation shall mean the exposure of a sample of interest to ionizing radiation, and the term radiosensitive shall refer to cells or individuals which display unusually adverse consequences after receiving moderate, or medically acceptable (i.e., nonlethal diagnostic or therapeutic doses), exposure to ionizing irradiation.

7 MmRad51 may perform a novel role in DNA replication, repair, or chromosomal disjunction. MmRAD51 expression is restricted during the cell cycle to late G.sub.1 /S/G.sub.2 and MmRAD51 expression was activated by mitogens that induced T and B cell proliferation suggesting a role in replication and repair (Yamamoto et al., 1996, 251:1-12). MmRad51 may take part in disjunction because it localizes to the kinetochores of diakinesis, and metaphase 1 chromosomes (Ashley et al., 1995).

8 The exact function or functions performed by MmRad51 are unimportant with regard to developing anti-proliferative drugs and cancer therapeutics as long as the disruption of the MmRad51 function provides a benefit to the patient. For the purposes of the present invention, it is assumed that the function of Rad51 is the repair of DSB; however, it is likely that Rad51 performs additional functions in the cell. However, it is important to note that at least some aspect of MmRad51 function is essential for cell proliferation and/or viability, and that molecules capable of disrupting MmRad51 function thus hinder cell proliferation or reduce cell viability. As such, any molecule that disrupts the MmRad51 pathway should prove useful for cancer therapy (for example).

9 Furthermore, disruption of any protein--protein interaction that involves either MmRad51 or any other molecule in the MmRad51 pathway should also prove useful for cancer therapy.

10 Protein--protein interactions are critical for recombinational repair in yeast cells, including interactions that involve ScRad51 and ScRad52 (Donovan et al., 1994; Milne et al., 1993). In addition, the human Rad51 and Rad52 proteins were shown to associate like their yeast homologues (Shen et al., 1996, J. Biol. Chem. 271:148-152).

11 To isolate proteins that associate with MmRad51, a yeast two-hybrid screen was

performed with MmRad51 as the "bait" and a T cell library and an embryonic cell library as the "prey". Among other proteins identified using this screen, MmRad51 and Brca2 were isolated, and the interactions identified using this screen may prove critical for in vivo function. Additional biochemical binding assays that may prove useful for identifying compounds that are able to associate with MmRad51 (or any other target protein) are well known in the art including, but not limited to: equilibrium or membrane flow dialysis, antibody binding assays, gel-shift assays, in vitro binding assays, filter binding assays, enzyme-linked immunoabsorbent assays (ELISA), western blots, co-immunoprecipitation, immunogold co-immunoprecipitation, coimmunolocalization, co-crystallization, fluorescence energy transfer, competition binding assays, chemical crosslinking, and affinity purification. In addition, genetic analysis may be used to identify accessory proteins that interact with MmRad51 or are peripherally involved in MmRad51 function. Where the MmRad51 accessory protein is essential to MmRad51 function, mutation in the genes encoding these proteins should typically result in phenotypes similar to those associated with MmRad51 mutations. Similarly, where the MmRad51 accessory proteins function to inhibit or retard MmRad51 activity, mutations in the genes encoding these factors shall generally mimic antagonist phenotypes.

12 The MmRad51 self-association was investigated further. Deletion analysis revealed that the MmRad51 self-association occurred in the N-terminal region which further demonstrated conservation of function with ScRad51 and RecA since both were shown to self-associate via the N-terminal region of the protein (Donovan et al., 1994; Horii, 1992; Story et al., 1992, 1993; Tateishi et al., 1992; Yarranton and Sedgwick, 1982). Although the presently described invention has been specifically exemplified using a species exemplary of the order mammalia, given the relatively high level of interspecies sequence similarity (and functional similarity) observed in the Rad51 proteins, it is clear that the present invention may be broadly applied to other mammalian species, including humans, as well as non-mammalian animals such as birds, and fish.

13 In addition to mice, examples of mammalian species that may be used in the practice of the present invention include, but are not limited to: humans, non-human primates (such as chimpanzees), pigs, rats (or other rodents), rabbits, cattle, goats, sheep, and guinea pigs.

14 Given the critical importance of mammalian Rad51 function, any disruption of the mammalian Rad51 or Rad52 complexes, or any member in their pathway will necessarily hinder cell proliferation or viability. When the Rad51 and Rad52 pathways were disrupted by introducing altered mouse rad51 into mouse cells, nonproductive protein--protein associations resulted. The altered forms of mouse rad51 were generated by disrupting a conserved nucleotide binding motif while preserving the protein association domain. The expression of these transgenes resulted in cellular toxicity. Presumably, the resulting nonproductive protein associations were responsible for the drastically reduced viability of these cells. In view of this result, it is clear one may reduce cell proliferation by disrupting mammalian Rad51 function, or the function of any protein in this repair pathway by hindering protein association by using defective proteins or other means such as small molecules.

15 Given that the Rad51 proteins are known to self-associate, the Rad51 protein sequence provides a template for the identification and genesis of peptides or factors that disrupt Rad51 function or activity For the purposes of the present invention a "peptide" is any sequence of at least about five amino acids up to about 100 amino acids. Typically, the peptides of the present invention can encompass enzymatic domains, DNA, RNA, or protein binding domains, or any fragment of a protein or amino acid sequence that directly or indirectly provide the desired function of disrupting cellular Rad51 or Rad52 activity. Accordingly, an additional embodiment of the present invention are peptides or polypeptides that correspond at least five contiguous amino acids of the mammalian Rad51 amino acid sequence (SEQ ID NO. 1), or the human Rad51 amino acid sequence (SEQ ID NO. 2) that retain the property of being capable of binding a mammalian Rad51 and/or inhibiting Rad51 function (as detected using a suitable biochemical, genetic, or cellular assay).

16 Additionally, the blocking of normal Rad51 function may induce programmed

17 cell death. Thus, one aspect of the present invention are a novel class of therapeutic agents, factors, or compounds that have been engineered, or are otherwise capable of disrupting the essential processes that are mediated by, or associated with, normal Rad51 or Rad52 activity. Accordingly, it is contemplated that this novel class of therapeutics agents may be used to treat diseases including, but not limited to, autoimmune disorders and diseases, inflammation, cancer, graft rejection, and any of a variety of proliferative or hyperproliferative disorders.

18 Typical examples of therapeutic agents based on the above presently described molecules include, but are not limited to, defective (either engineered or naturally occurring) forms of the proteins that associate with the protein complexes, inhibitory fragments of the proteins, wild type and altered genes that code for proteins that disrupt mammalian Rad51 function, small organic molecules, antisense nucleic acid sequences, oligonucleotides that inhibit expression or activity via a triplex mechanism, peptides, aptameric oligonucleotides, and the like.

19 More particularly, examples of engineered proteins may include, but are not limited to, proteins that comprise inactivating mutations in conserved active sites (e.g., ATP binding motifs, DNA or protein binding domains, catalytic sites, etc.), fusion proteins that comprise at least one inhibitory domain, and the like.

20 The above agents may be obtained from a wide variety of sources. For example, standard methods of organic synthesis may be used to generate small organic molecules that mimic the desired regions of the target DNA repair proteins. In addition, combinatorial libraries comprising a vast number of compounds (organic, peptide, or nucleic acid, reviewed in Gallop et al. 1994, J. Med. Chem. 37(9):1233-1251; Gordon et al., 1994, J. Med. Chem. 37(10):1385-1401; and U.S. Pat. No. 5,424,186 all of which are herein incorporated by reference) may be screened for the ability to bind and inhibit the activity of proteins involved in DSB repair or any other potential mammalian Rad51 function.

21 In particular, inhibitory peptides should prove very useful. Such compounds may include, but are not limited to, peptides such as, for example, soluble peptides, including but not limited to members of random peptide libraries; (see, e.g., Lam et al., 1991, Nature 354:82-84; Houghten et al., 1991, Nature 354:84-86), and combinatorial chemistry-derived molecular library made of D- and/or L- configuration amino acids, phosphopeptides (including, but not limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang et al., 1993, Cell 72:767-778).

22 Given that an important aspect of DSB repair is the interaction of proteins, additional aspects of the invention are the use of screening assays to detect interactions or the lack of such interactions of proteins involved in DSB repair. The following assays are designed to identify compounds that interact with (e.g., bind to) proteins involved in DSB repair. The compounds which may be screened in accordance with the invention include but are not limited to peptides, antibodies and fragments thereof, prostaglandins, lipids and other organic compounds (e.g., terpines, peptidomimetics) that bind to or mimic the activity triggered by a natural ligand (i.e., agonists) or inhibit the activity triggered by a natural ligand (i.e., antagonists) of a protein involved in DSB repair; as well as peptides, antibodies or fragments thereof, and other organic compounds that mimic the natural ligand for a given protein involved in DSB repair.

23 Such compounds may include, but are not limited to, peptides such as, for example, soluble peptides, including but not limited to members of random peptide libraries (see, e.g., Lam, K. S. et al., 1991, Nature, 354:82-84; Houghten, R. et al., 1991, Nature, 354:84-86), and combinatorial chemistry-derived molecular library peptides made of D- and/or L- configuration

amino acids, phosphopeptides (including, but not limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell, 72:767-778); antibodies (including, but not limited to, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain =antibodies, and FAb, F(ab).sub.2 and FAb expression library fragments, and epitope-binding fragments thereof); and small organic or inorganic molecules.

24 Other compounds which can be screened in accordance with the invention include but are not limited to small organic molecules that are able to gain entry into an appropriate cell and affect DSB repair by, for example, modulating protein--protein interactions important for DSB repair (e.g., by interacting with a protein involved in DSB repair); or such compounds that affect the activity of a gene encoding a protein involved in DSB repair.

25 Computer modeling and searching technologies permit identification of compounds, or the improvement of already identified compounds, that can modulate DSB repair by, for example, modulating protein--protein interactions involved in DSB repair. Having identified such a compound or composition, the active sites or regions are identified. Such active sites might typically be the binding partner sites, such as, for example, the interaction domains of a protein important for DSB repair with its cognate ligand. The active site can be identified using methods known in the art including, for example, from the amino acid sequences of peptides, from the nucleotide sequences of nucleic acids, or from study of complexes of the relevant compound or composition with its natural ligand. In the latter case, chemical or X-ray crystallographic methods can be used to find the active site by finding where on the factor the complexed ligand is found.

26 Next, the three dimensional geometric structure of the active site is determined. This can be done by known methods, including X-ray crystallography, which can determine a complete molecular structure. On the other hand, solid or liquid phase NMR can be used to determine certain intra-molecular distances. Any other experimental method of structure determination can be used to obtain partial or complete geometric structures. The geometric structures may be measured with a complexed ligand, natural or artificial, which may increase the accuracy of the active site structure determined.

27 If an incomplete or insufficiently accurate structure is determined, the methods of computer based numerical modeling can be used to complete the structure or improve its accuracy. Any recognized modeling method may be used, including parameterized models specific to particular biopolymers such as proteins or nucleic acids, molecular dynamics models based on computing molecular motions, statistical mechanics models based on thermal ensembles, or combined models. For most types of models, standard molecular force fields, representing the forces between constituent atoms and groups, are necessary, and can be selected from force fields known in physical chemistry. The incomplete or less accurate experimental structures can serve as constraints on the complete and more accurate structures computed by these modeling methods.

28 Finally, having determined the structure of the active site, either experimentally, by modeling, or by a combination thereof, candidate modulating compounds can be identified by searching databases containing compounds along with information on their molecular structure. Such a search seeks compounds having structures that match the determined active site structure and that interact with the groups defining the active site. Such a search can be manual, but is preferably computer assisted. The compounds found from such a search generally identify modulating compounds, or genes encoding the same, that are selected for further study or gene targeting.

29 Alternatively, these methods can be used to identify improved modulating compounds from an already known modulating compound or ligand. The composition of the known compound can be modified and the structural effects of modification can be determined using the experimental and computer modeling methods described above applied to the new composition. The altered structure

is then compared to the active site structure of the compound to determine if an improved fit or interaction results. In this manner systematic variations in composition, such as by varying side groups, can be quickly evaluated to obtain modified modulating compounds or ligands of improved specificity or activity.

30 Further experimental and computer modeling methods useful to identify modulating compounds based upon identification of the active sites of regulatory protein interactions, and related transduction factors will be apparent to those of skill in the art.

31 Representative examples of molecular modeling systems include the CHARMm and QUANTA programs (Polygen Corporation, Waltham, Mass.). CHARMm performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modeling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

32 Although described above with reference to design and generation of compounds which could alter binding, one could also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins, for compounds which are inhibitors or activators of the proteins and genes that are important for any aspect of DSB repair.

33 In vitro systems may be designed to identify compounds capable of interacting with (e.g., binding to) the regulatory proteins identified using the subject methods. The identified compounds may be useful, for example, in modulating the activity of wild type and/or mutant proteins important for DSB repair. In vitro systems may also be utilized to screen for compounds that disrupt normal interactions important for DSB repair.

34 The assays used to identify compounds that bind to proteins important for DSB repair involve preparing a reaction mixture of a given protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex which can be removed and/or detected in the reaction mixture. The protein used can vary depending upon the goal of the screening assay. For example, where agonists of the natural ligand are sought, a full length protein, or a fusion protein containing a protein or polypeptide that affords advantages in the assay system (e.g., labeling, isolation of the resulting complex, etc.) can be utilized.

35 The screening assays can be conducted in a variety of ways. For example, one method to conduct such an assay would involve anchoring the protein, polypeptide, peptide or fusion protein or the test substance onto a solid phase and detecting binding between the protein and test compound. In one embodiment of such a method, the protein reactant may be anchored onto a solid surface, and the test compound, which is not anchored, may be labeled, either directly or indirectly. In another embodiment of the method, the test protein is anchored on the solid phase and is complexed with a labeled antibody (and where a monoclonal antibody is used, it is preferably specific for a given region of the protein). Then, a test compound could be assayed for its ability to disrupt the association of the protein/antibody complex.

36 In practice, microtiter plates, or any modernized iteration thereof, may conveniently be utilized as the solid phase. The anchored component may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized antibody, preferably a monoclonal antibody, specific for the protein to be immobilized may be used to anchor the protein to the solid surface. The surfaces may be prepared in advance and stored.

37 In order to conduct the assay, the nonimmobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface.

The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously nonimmobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously nonimmobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the previously nonimmobilized component (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody).

38 Alternatively, a reaction can be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized antibody specific for the test protein, polypeptide, peptide or fusion protein, or the test compound to anchor any complexes formed in solution, and a labeled antibody specific for the other component of the possible complex to detect anchored complexes.

39 Macromolecules that interact with a given protein important for DSB repair are referred to, for purposes of this discussion, as "binding partners". Therefore, it is desirable to identify compounds that interfere with or disrupt the interaction with such binding partners which may be useful in modulating DSB repair.

40 The basic principle of the assay systems used to identify compounds that interfere with the interaction between a protein and its binding partner or partners involves preparing a reaction mixture containing the test protein, polypeptide, peptide or fusion protein as described above, and the binding partner under conditions and for a time sufficient to allow the two to interact and bind, thus forming a complex. In order to test a compound for inhibitory activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound may be initially included in the reaction mixture, or may be added at a time subsequent to the addition of the test protein and its binding partner. Control reaction mixtures are incubated without the test compound or with a placebo.

41 The formation of any complexes between the test protein and the binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the test protein and the binding partner.

42 The assay for compounds that interfere with protein binding can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the test protein or the binding partner onto a solid phase and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. The examples below describe similar assays which may be easily modified to screen for compounds which disrupt or enhance the interaction. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction by competition can be identified by conducting the reaction in the presence of the test substance; i.e., by adding the test substance to the reaction mixture prior to or simultaneously with the test protein and interactive binding partner. Alternatively, test compounds that disrupt preformed complexes, e.g., compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are described briefly below.

43 In a heterogeneous assay system, either the test protein, or the interactive binding partner, is anchored onto a solid surface, while the non-anchored species is labeled, either directly or indirectly. In practice, microtiter plates are conveniently utilized. The anchored species may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished simply by coating the solid surface with a solution of the test protein or binding partner and drying. Alternatively, an immobilized antibody

specific for the species to be anchored may be used to anchor the species to the solid surface. The surfaces may be prepared in advance and stored.

44 In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the

45 reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes can be detected.

46 Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds which inhibit complex or which disrupt preformed complexes can be identified.

47 In an alternate embodiment of the invention, a homogeneous assay can be used. In this approach, a preformed complex of the test protein and the interactive binding partner is prepared in which either protein is labeled, but the signal generated by the label is quenched due to formation of the complex (see, e.g., U.S. Pat. No. 4,109,496 by Rubenstein which utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances which disrupt the binding interaction can be identified.

48 For an example of a typical labeling procedure, a test protein or a peptide fragment, e.g., corresponding to the relevant binding domain, can be fused to a glutathione-S-transferase (GST) gene using a fusion vector, such as pGEX-5X-1, in such a manner that its binding activity is maintained in the resulting fusion protein. The interactive binding partner can be labeled with radioactive isotope, for example, by methods routinely practiced in the art. In a heterogeneous assay, e.g., the GST-fusion protein can be anchored to glutathione-agarose beads. The interactive binding partner can then be added in the presence or absence of the test compound in a manner that allows interaction and binding to occur. At the end of the reaction period, unbound material can be washed away. The interaction between the fusion product and the labeled interactive binding partner can be detected by measuring the amount of radioactivity that remains associated with the glutathione-agarose beads. The successful inhibition of binding by the test compound will result in a decrease in measured radioactivity.

49 Alternatively, the GST-fusion protein and the labeled interactive binding partner can be mixed together in liquid in the absence of the solid glutathione-agarose beads. The test compound can be added either during or after the species are allowed to interact. This mixture can then be added to the glutathione-agarose beads and unbound material is washed away. Again the extent of binding inhibition can be measured by determining the amount of radioactivity associated with the beads.

50 In another embodiment of the invention, these same techniques can be employed using peptide fragments that correspond to the binding domains of the test proteins, in place of the full length proteins. Any number of methods routinely

practiced in the art can be used to identify and isolate the binding sites. These methods include, but are not limited to, mutagenesis of the gene encoding the protein and screening for disruption of binding in a co-immunoprecipitation assay. Sequence analysis of the gene encoding the protein will reveal the mutations that correspond to the region of the protein involved in interactive binding.

51 The invention encompasses cell-based and animal model-based assays for the identification of compounds exhibiting the ability to alter or correct phenotypes associated with the various genotypes identified and constructed using the present methods. Such cell-based assays can also be used as the standard to assay for purity and potency of the compounds, including recombinantly or synthetically produced proteins or compounds.

52 Given that they will serve as templates for the rational design of agents for disrupting DSB repair activity in the cell, it would be advantageous to purify each of the individual proteins that are directly or indirectly involved in DSB repair of any other potential mammalian Rad51 function. The various proteins involved in the DSB repair pathways may be purified using any of a number of variations of well established biochemical, and molecular biology techniques. Such techniques are well known to those of ordinary skill in the biochemical arts and have been extensively described in references such as Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology, Volume 152, Academic Press, San Diego, Calif. (1987; Molecular Cloning: A Laboratory Manual, 2d ed., Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989); Current Protocols in Molecular Biology, John Wiley & Sons, all Vols., 1989, and periodic updates thereof); New Protein Techniques: Methods in Molecular Biology, Walker, J. M., ed., Humana Press, Clifton, N.J., 1988; and Protein Purification: Principles and Practice, 3rd. Ed., Scopes, R. K., Springer-Verlag, New York, N.Y., 1987. In general, techniques including, but not limited to, ammonium sulfate precipitation; centrifugation, ion exchange, gel filtration, and reverse-phase chromatography (and the HPLC or FPLC forms thereof) may be used to purify the various proteins of the DSB repair complex.

53 Additionally, purified preparations of the presently described DNA repair proteins, associated proteins, or fragments thereof, may be used to generate antisera specific for a given agent. Accordingly, additional embodiments of the present invention include polyclonal and monoclonal antibodies that recognize epitopes of the presently described DNA repair complex proteins. The factors used to induce the antibodies of interest need not be biologically active; however, the factors should induce immunological activity in the animal used to generate the antibodies.

54 Given that similar methodologies may be applied to the generation of antibodies to the various factors, for purposes of convenience, only the Rad51 factor antibodies will be discussed further.

55 Polypeptides for use in the induction of Rad51-specific antibodies may have an amino acid sequence consisting of at least three amino acids, and preferably at least 10 amino acids, that mimic a portion of the amino acid sequence of Rad51, and may contain the entire amino acid sequence of naturally occurring Rad51 or a Rad51-derivative.

56 Anti-Rad51 antibodies are expected to have a variety of medically useful applications, several of which are described generally below. More detailed and specific descriptions of various uses for anti-Rad51 antibodies are provided in the sections and subsections which follow. Briefly, anti-Rad51 antibodies may be used for the detection and quantification of Rad51 polypeptide expression in cultured cells, tissue samples, and in vivo. Such immunological detection of Rad51 may be used, for example, to identify, monitor, and assist in the prognosis of neoplasms that have been treated with factors that inhibit DSB repair. Additionally, monoclonal antibodies recognizing epitopes from different parts of the Rad51 structure may be used to detect and/or distinguish between native Rad51 and various subcomponent and/or mutant forms of the molecule. Additionally, anti-Rad51 monoclonal antibodies may be used to test preparations

of agents or factors that mimic segments of Rad51, or are designed to impair protein association with Rad51, or to competitively inhibit DNA binding. In addition to the various diagnostic and therapeutic utilities of anti-Rad51 antibodies, a number of industrial and research applications will be obvious to those skilled in the art, including, for example, the use of anti-Rad51 antibodies as affinity reagents for the isolation of Rad51-associated polypeptides, and as immunological probes for elucidating the biosynthesis, metabolism and biological functions of Rad51. Rad51 antibodies may also be used to purify Rad51 or Rad51-associated factors by affinity chromatography.

57 Once purified, the proteins of interest may be partially sequenced, and these data may be used to design degenerate oligonucleotide probes for use in cloning the genes encoding the various proteins that are associated with DSB repair. Alternatively, any of a variety of public or private sequence data bases may be searched for nucleic acid or peptide sequences that share homology with genes and proteins associated with Rad51-mediated DSB repair. Once a similar sequence is identified, peptides may be produced and screened for inhibitory activity. Where a nucleic acid library is involved, one could synthesize a probe corresponding to the nucleic acid sequence of interest, and use the probe to clone a full-length version of the corresponding gene (if necessary). Accordingly, an additional embodiment of the presently claimed invention are nucleic acid sequences that are capable of hybridizing to sequences encoding the proteins that are associated with DSB repair under stringent conditions. For the purposes of the present invention, the term "stringent conditions" generally refers to hybridization conditions that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50.degree. C.; or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42.degree. C.; or (3) employ 50 formamide, 5.times. SSC (0.75 M NaCl, 0.075 M Sodium pyrophosphate, 5.times. Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42.degree. C., with washes at 42.degree. C. in 0.2.times. SSC and 0.1% SDS. The above examples of hybridization conditions are merely provided for purposes of exemplification and not limitation. A more thorough treatise of the such routine molecular biology techniques may be found in Sambrook et al., Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, Vols. 1-3: (1989), and periodic updates thereof, herein incorporated by reference.

58 Once isolated, the genes encoding the proteins involved in DSB repair may be recombinantly expressed using standard vectors and hosts. Examples of vectors that may be used to express proteins of interest are provided in Sambrook et al., Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, Vols. 1-3: (1989). In particular, eucaryotic viruses may be used as vectors to transduce any of a wide variety of plant and animal cells to overexpress the desired proteins. Examples of such viruses include, but are not limited to, adenovirus, papilloma virus, herpes virus, adeno-associated virus, rabies virus, bacculo virus, retrovirus, plant viruses, and the like (See generally, Sambrook et al., Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, Vol. 3:16.1-16.89 (1989); U.S. Pat. No. 5,316,931, issued May 31, 1994, herein incorporated by reference).

59 Preferably, agents that disrupt DSB repair shall be substantially specific for blocking the desired repair pathways. For the purposes of the present invention, the term substantially specific shall mean that a given agent is capable of being dosaged to provide the desired effect while not causing undue cellular toxicity.

60 One of ordinary skill will appreciate that, from a medical practitioner's or patient's perspective, virtually any alleviation or prevention of an undesirable symptom (e.g., symptoms related to disease, sensitivity to environmental factors, normal aging, and the like) would be desirable. Thus, for the purposes of this Application, the terms "treatment", "therapeutic use", or "medicinal use", used herein shall refer to any and all uses of compositions

comprising the claimed agents which remedy a disease state or symptoms, or otherwise prevent, hinder, retard, or reverse the progression of disease or other undesirable symptoms in any way whatsoever.

61 When used in the therapeutic treatment of disease, an appropriate dosage of presently described agents, or derivatives thereof, may be determined by any of several well established methodologies. For instance, animal studies are commonly used to determine the maximal tolerable dose, or MTD, of bioactive agent per kilogram weight. In general, at least one of the animal species tested is mammalian. Those skilled in the art regularly extrapolate doses for efficacy and avoiding toxicity to other species, including human. Before human studies of efficacy are undertaken, Phase I clinical studies in normal subjects help establish safe doses.

62 Additionally, the bioactive agents may be complexed with a variety of well established compounds or structures that, for instance, enhance the stability of the bioactive agent, or otherwise enhance its pharmacological properties (e.g., increase in vivo half-life, reduce toxicity, etc.).

63 Another aspect of the present invention includes formulations that provide for the sustained release of DSB repair antagonists. Examples of such sustained release formulations include composites of biocompatible polymers, such as poly(lactic acid), poly(lactic-co-glycolic acid), methylcellulose, hyaluronic acid, collagen, and the like. The structure, selection and use of degradable polymers in drug delivery vehicles have been reviewed in several publications, including, A. Domb et al., Polymers for Advanced Technologies 3:279-292 (1992). Additional guidance in selecting and using polymers in pharmaceutical formulations can be found in the text by M. Chasin and R. Langer (eds.), "Biodegradable Polymers as Drug Delivery Systems, " Vol. 45 of "Drugs and the Pharmaceutical Sciences," M. Dekker, New York, 1990. Liposomes may also be used to provide for the sustained release of DSB repair antagonists. Details concerning how to use and make liposomal formulations of drugs of interest can be found in, among other places, U.S. Pat. No 4,944,948; U.S. Pat. No. 5,008,050; U.S. Pat. No. 4,921,706; U.S. Pat. No. 4,927,637; U.S. Pat. No. 4,452,747; U.S. Pat. No. 4,016,100; U.S. Pat. No. 4,311,712; U.S. Pat. No. 4,370,349; U.S. Pat. No. 4,372,949; U.S. Pat. No. 4,529,561; U.S. Pat. No. 5,009,956; U.S. Pat. No. 4,725,442; U.S. Pat. No. 4,737,323; U.S. Pat. No. 4,920,016. Sustained release formulations are of particular interest when it is desirable to provide a high local concentration of DSB repair antagonist, e.g., near a tumor, site of inflammation, etc.

64 Where diagnostic, therapeutic or medicinal use of the presently described agents, or derivatives thereof, is contemplated, the bioactive agents may be introduced in vivo by any of a number of established methods. For instance, the agent may be administered by inhalation; by subcutaneous (sub-q); intravenous (I.V.), intraperitoneal (I.P.), or intramuscular (I.M.) injection; or as a topically applied agent (transdermal patch, ointments, creams, salves, eye drops, and the like).

65 Additionally, an alternative means for employing the presently disclosed anti-proliferation agents includes the use of vectors to directly insert genes encoding the agents into target cells (e.g., gene therapy). For example, when the tumor cells express the genes encoding the desired sequences, DSB repair will be disrupted and the tumor cell will die. Alternatively, one could attack tumor cells using a strategy conceptually similar to that disclosed in U.S. Pat. No. 5,529,774 herein incorporated by reference. In brief, cells that produce transducing virus encoding sequence that disrupts DSB repair may be implanted at or near the tumor mass. As the producer cells continue to elaborate virus, the growing tumor cells are infected and effectively killed as they express the agent that blocks DSB repair. The above methodology has proven useful in the treatment of glioblastomas and other tumors of the brain by using retroviral vectors to selectively target actively replicating tumor cells. A similar methodology could be used to deliver antisense sequences that target (and thus inhibit) the expression of Rad51 or any of the proteins involved in the Rad51 or Rad52 pathways.

66 The mammalian Rad.sub.51 or Rad52-mediated repair pathways, and the associated proteins, are essential for cell proliferation or viability. These DNA repair pathways most likely function by repairing DSB via homologous recombination between sister chromatids during S/G.sub.2 (recombinational repair); however, during G.sub.1, the repair of DSB may also occur via nonhomologous recombination (nonhomologous end joining).

67 The nonhomologous recombination pathway was once thought to be the major repair pathway in mammalian cells. Much of this belief stems from gene targeting data that demonstrated homologous recombination to be less frequent than random or illegitimate recombination (Bradley et al., 1992, Bio/Technology 10:534-39). Other data demonstrated that chromosomal DSB frequently were joined without homology or with only very short stretches of homology (Rouet and Jasin, 1994, Mol. Cell. Biol. 14:8096-8105). DNA-dependent protein kinase (DNA-PK) is critical for nonhomologous but not homologous repair of DSB (Liang et al., 1996, Proc. Natl. Acad. Sci. USA 93:8929-33). A biphasic response to ionizing radiation was observed in DNA-PK-deficient cell lines with resistance in late S phase suggesting that DNA-PK functions in G.sub.1 and another repair pathway functions in S phase (Jeggo, 1990, Mutation Research 239:1-16). DNA-PK is composed of a catalytic subunit called DNA-PK.sub.cs and a DNA end-binding subunit called Ku which is a heterodimer of Ku70 and Ku86 (Park et al., 1996, J. Biol. Chem. 1996:18996-19000, for review, see Roth et al., 1995; Shen et al., 1996. Analysis 30 of DNA-PK activity has come from scid (severe combined immunodeficient) mice which are deficient in DNA-PK.sub.cs (Kirchgessner et al., 1995, Science 267:1178-82), and Ku86-deficient mice (Nussenzweig et al., 1996, Nature 382:551-55; Zhu et al., 1996, Cell 86:379-89). Both scid and Ku86-deficient mice are immune deficient due to a defect in repair of DSB generated during V(D)J recombination. Unfortunately, it is impossible to analyze V(D)J recombination in rad51-mutant mice or cells; however, it is unlikely that MmRad51 plays a role in this process since MmRad51 localizes to the nucleus in late G.sub.1 through G.sub.2 (Yamamoto et al., 1996, 251:1-12), and V(D)J recombination occurs in G.sub.0 /G.sub.1 (Schlissel et al., 1993, Genes & Dev. 7:2520-32). In general, scid and Ku86-deficient cells do have similarities to MmRad51-deficient cells. All are hypersensitive to ionizing radiation, and Ku86-deficient cells were prematurely senescent in tissue culture, indicating a similar function. However, since scid and Ku86-deficient mice and cells were viable and MmRad51-deficient cells were not, the consequences of removing the putative homologous recombination pathway to repair DSB appears to be more vital than the removal of the nonhomologous pathway.

68 The presently described DSB repair antagonists are particularly deemed useful for the treatment of cancer. Cancers that may be treated by the methods of the invention include, but are not limited to: Cardiac: sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma; Lung: bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma; Gastrointestinal: esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma); Genitourinary tract: kidney (adenocarcinoma, Wilm's tumor [nephroblastoma], lymphoma, leukemia), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma); Liver: hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastom, angiosarcoma, hepatocellular adenoma, hemangioma; Bone: osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum

cell sarcoma), multiple myeloma, malignant giant cell tutrr, chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors; Nervous system: skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma [pinealoma], glioblastoma multiforme, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), spinal cord (neurofibroma, meningioma, glioma, sarcoma); Gynecological: uterus (endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma, [serous cystadenocarcinoma, mucinous cystadenocarcinoma, endometrioid tumors, celioblastoma, clear cell carcinoma, unclassified carcinoma], granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma [embryonal rhabdomyosarcoma], fallopian tubes (carcinoma); Hematoloqic: blood (myeloid leukemia [acute and chronic], acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic syndrome), Hodgkin's disease, non-Hodgkin's lymphoma [malignant lymphoma]; Skin: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles, dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis; and Adrenal glands: neuroblastoma.

69 In addition to cancer, the presently disclosed compounds are effective against any of a wide variety of hyperproliferative disorders including, but not limited to: autoimmune disease, arthritis, inflammatory bowel disease, proliferation induced after medical procedures, including, but not limited to, surgery, angioplasty, and the like.

70 The anti-cancer application of agents that functionally disrupt mammalian Rad51, Rad52 or any member in the DSB repair pathway, requires that DSB repair remains equally critical in cancer cells. Cancer cells lack many of the normal cell cycle regulatory mechanisms that are critical to controlling proliferation, and inducing programmed cell death, and it remains possible that the absence of these mechanisms renders Rad51 and/or Rad52 function nonessential. The protein p53 is central to regulation of the cell cycle, and stimulation of cell death in response to DNA damage including DNA damaged by ionizing radiation (reviewed by Ko and Prives, 1996, Genes & Develop. 10:1054-72). p53 is the most commonly mutated gene in cancer cells (Donehower et al., 1992, Nature 356:215-21; Vogelstein, 1990, Nature 348:681-682) and mutations in p53 are known to increase cell proliferation and promote chromosomal instability (Harvey et al., 1993, Oncogene 8:2457-67).

71 The early lethal phenotype in rad51.sup.M1 mutant embryos and cells may be stimulated by a cell cycle response to unrepaired DNA damage. DNA damage was shown to inhibit progression through the cell cycle, demonstrating a relationship between DNA lesions and cell cycle proteins (Carr and Hoekstra, 1995, Trends in Cell Biology 5:32-40). In mitotically dividing budding yeast cells, a single DSB in a dispensable plasmid was sufficient to induce cell death, partly under the control of Rad9 (Bennett et al., 1993, Proc. Natl. Acad. Sci. USA 90:5613-17; Schiest1 et al., 1989, Mol. Cell. Biol. 9:1882-9654, Weinert and Hartwell, 1988, Science 241:317-22). In mammalian cells, the tumor suppressor gene, p53, responded to DNA damage induced by .gamma.-radiation by delaying the cell cycle, or inducing programmed cell death (Kastan et al., 1991, Cancer Research 51:6304-11; Kuerbitz et al., 1992, Proc. Natl. Acad. Sci. USA 89:7491-95). These responses may be the critical tumor suppressor function of p53 (Baker et al., 1990, Science 249:912-15; Lowe et al., 1994, Science 266:807-10, Symonds et al., 1994, Cell 78:703-11). Induction of p53 after exposure to ionizing radiation and restriction endonuclease suggest that the formation of DSB may initiate a p53 response (Lu and Lane, 1993, Cell 75:765-78).

72 p53 was at least partly responsible for regulating the rad51.sup.M1 phenotype because development was extended from the early egg cylinder stage to the head fold stage in a p53-mutant background. However, the double-mutant embryos died

from either accumulation of DNA damage resulting in metabolic incompetence and mitotic failure, or p53-independent regulation. Murine embryonic fibroblasts, generated from double-mutant embryos, failed to proliferate and were completely senescent in tissue culture; thus, demonstrating that MmRad51 function was critical in cells that exhibit chromosomal instability and accelerated proliferation. It is therefore likely that disruption of MmRad5 or any other protein in its pathway or disruption of any protein--protein interaction important in the DSB repair pathway results in reduced proliferation or decreased cell viability. This feature remains true even in cells with reduced capacity to regulate the cell cycle.

73 The present invention is further illustrated by the following examples, which are not intended to be limiting in any way whatsoever.

74 5.0. EXAMPLES

75 5.1. Cloning of the Mouse MmRAD51 cDNA

76 The MmRAD51 cDNA sequence was cloned and used to generate an expression vector. The 5' end of cDNA was amplified by RT-PCR from mouse testis RNA and was then used as a probe to screen a mouse brain cDNA library. One clone was identified and sequenced. The coding sequence was identical to MmRAD51 disclosed in published reports (Morita et al., 1993; Shinohara et al., 1993); however, the clone contained about 300 additional base pairs of 5' noncoding sequence and about 400 extra base pairs of 3' noncoding sequence (FIG. 1).

77 5.2. The Use of a Yeast Two-Hybrid Screen to Isolate Proteins That Associate with MmRad51

78 ScRad51 was shown to self-associate as well as associate with other proteins such as ScRad52 and ScRad55 (Donovan et al., 1994; Hays et al., 1995; Johnson and Symington, 1995; Milne and Weaver, 1993; Shinohara et al., 1992). Kluyveromyeces lactis RAD51 and RAD52 did not rescue a rad51.DELTA. rad52.DELTA. strain of S. cerevisiae and overexpression of ScRAD51 suppressed rad55 and rad57 mutant yeast which indicates interacting proteins are necessary (Donovan et al., 1994; Hays et al., 1995). Also, Dmc1 and ScRad51 colocalized to the synaptonemal complex which suggested that they act together during meiotic recombination (Bishop, 1994).

79 The modified yeast two-hybrid system was used to isolate proteins that associate with mammalian Rad51 which is a genetic screen for determining protein--protein interactions (Harper et al., 1993). One of the proteins is a hybrid of the GAL4 DNA-binding domain fused to MmRad51 (the "bait"). The other is a hybrid of the GAL4 transactivating domain fused to an embryonic or a T cell cDNA library (the "prey"). The bait and prey were co-expressed in HF7c yeast that contained two reporters, HIS3 and lacZ fused to the GAL4 promoter and grown in media lacking histidine and containing 25 mM 3-AT (an antimetabolite; 3-amino-1,2,4-triazole)-Functional GAL4 was created when the DNA binding domain and the transactivation domain were juxtaposed, ideally by a MmRad51-protein interaction. Such an interaction induced the HIS3 and lacZ genes allowing a positive colony to survive in medium lacking histidine and to turn blue in X-Gal (5-bromo-4-chloro-3-indolyl-.beta.-D-galactosidase).

80 Seven specific clones were isolated from this screen. A 13.5 day embryonic cDNA library (500 .mu.g) was transfected into 5.times.10.sup.6 cells and plated onto forty 15 cm plates. A T cell cDNA library (400 .mu.g) was transfected into 4.times.10.sup.6 cells and plated onto twenty 15 cm plates. A total of 80 His.sup.+ colonies grew in about 3 days. Of these, 40 turned blue after about 5 to 30 minutes of exposure to X-gal. These colonies were tested for specificity by transfecting HF7c cells without bait or with a nonspecific bait (E12). Nonspecific associations were observed in 20 clones. The inserts in the other clones were sequenced and 13 were out of frame and seven were in frame. The sequences for the remaining seven clones were screened in the GCG data base. Homologues were found for four clones and three clones were novel (Table 1). The protein produced by clone 1 was 100% homologous to MmRad51 which showed

that the screen was successful because RecA and ScRad51 are both known to self-associate. The protein produced from clone 2 was 100% homologous to a metal response element binding protein, M96 (Inouye et al., 1994, DNA and Cell Biol. 13(7):731-742). The function of M96 is unknown. The protein produced from clone 3 was 48% homologous to human XP-G (ERCC-5) and 45% homologous to chicken Histone Hi. A mutation in XP-G is responsible for the genetic disorder xeroderma pigmentosum (Cleaver, 1994; Cleaver and Kraemer, 1995, In The metabolic basis of inherited disease, p. 4393-4419, 7th ed. McGraw-Hill, New York.). XP-G is a homologue of the S. cerevisiae excision repair protein, ScRad2 which is a ssDNA endonuclease. It is possible that MmRad51 repairs single-strand breaks as well as double-strand breaks and that single-strand breaks can initiate recombination. Histone H1 is a component of the nucleosome and comprises a group of related proteins that vary in tissues and are poorly conserved across species. The length of DNA may be affected by Histone Hi binding to the linker region and joining adjacent nucleosomes. The protein produced from clone 4 was 100% homologous to the human breast cancer gene, BRCA2 (Tavtigian et al., 1996, Nat. Gen. 12:333-337; Wooster et al., 1995, Nature 378:789-792). The function of Brca2 is unknown; however, like p53, it is a tumor suppressor gene and may therefore regulate the cell cycle in response to DNA damage. Thus, the observed association with a DNA repair gene, MmRad51, is consistent with such an activity.

TABLE 1

Clones isolated from the yeast two-hybrid screen

Clone	Homology	Library
1	100% to MmRad51	T cell
2	100% to M96	embryo
3	45% to Histone H1, 48% to XP-G	embryo
4	100% to Brca2	T cell
5	novel	T cell
6	novel	embryo
7	novel	embryo

81 Clones isolated from a yeast two-hybrid screen with MmRad51 as the "bait" and an embryonic or T cell cDNA library as the "prey". The inserts obtained from the prey were sequenced and compared to sequences in the GCG data base. The measured extent of protein homology is listed. All clones strongly associated with MmRad51 in the N-terminal region (amino acids 1-43). Colonies grew within three days in 3-AT, and cells generally stained blue after about 5 minutes of X-gal exposure.

82 5.3. Deletion Analysis of MmRad5 to Isolate the Protein Association Region

83 A deletion analysis was performed to isolate the MmRad51 self-association domain. Full length MmRAD51 was used as the bait and deletions of 51RAD51 were the prey (FIG. 2). The "prey" MmRad51 deletions were individually co-transfected with the bait into HF7c cells. The relative levels of .beta.-galactosidase activity were measured for the MmRad51 deletion proteins as compared to full length MmRad51 which was considered to have 100% activity. Expression of the C-terminal region, TR43-339 and TR131-339 did not result in blue yeast cells after 10 hours, and the relative .beta.-galactosidase activity was about 1, or the same as for the nonspecific bait, E12. However, expression of the N-terminal region, TR1-43, stained yeast cells blue in less than 5 minutes and the relative .beta.-galactosidase activity was 43%. Interestingly, a sequence containing more of the N-terminal region of the protein, TR1-93, caused the yeast cells to stain blue after about 30 minutes of X-gal exposure, and reduced the relative .beta.-galactosidase activity to about 4%. In similar experiments, TR1-131 and TR1-175 respectively displayed 11% and 9% of the .beta.-galactosidase activity of the positive control. Nevertheless, these data

indicated that the N-terminal region was responsible for MmRad51 self-association. It also appeared that amino acids 43-93 inhibited self-association and that this inhibition was relieved by adding more of the C-terminal region of the protein. These

84 data indicated that MmRad51 was functionally conserved with ScRad51 since the self-association domain was also in the N-terminal region for both proteins even though these regions did not display conserved amino acid sequences.

85 The other six proteins listed in Table 1 were tested to determine if they interacted with the N-terminal region of MmRad51. All six strongly interacted with TR1-43; thus, the most N-terminal 43 amino acids were responsible for all the MmRad51 protein--protein interactions observed. Given the high level of homology shared between the human and murine Rad51 proteins (in the important N-terminal self-association region, the proteins only differ at amino acid positions 10 and 46 where the human sequence respectively contains an asparagine in lieu of the serine, and a phenylalanine in place of the tyrosine encoded by the mouse protein--both relatively conservative replacements), the presently described results should reflect the results expected from similar studies using the human Rad51 protein.

86 5.4. Transfection of Mouse Embryonic Stem Cells with Altered Alleles of Mammalian rad51

87 Both MmRad51 and ScRad51 self-associate using their respective N-terminal regions. This observation supports the hypothesis that these proteins remain functionally conserved. Functional conservation was further tested in the RecA core homology domain. In ScRad51, the RecA core homology region was shown to be essential for the repair of DSB. The gene rad51K-A191 was altered in the first ATP-binding motif, and a conserved Lysine was changed to an Alanine. The expression of rad51K-A191 in wild-type yeast cells dominantly impaired the repair of DNA damage and generated a rad51 null phenotype. Nonproductive protein--protein interactions were probably responsible for the dominant negative phenotype because rad51K-A191 was shown to associate with wild-type ScRad51 and ScRad52. If the MmRad51 structural domains were similar to ScRad51, then disruption of the conserved Lysine in the first ATP-binding motif should result in a null phenotype because of the nonfunctional associations with wild-type MmRad51 or other proteins in this pathway such as mouse Rad52 or Brca2. A null rad51 mutation resulted in a severe cell proliferation defect that prevented propagation of mutant mouse cells in tissue culture. Therefore, cells that expressed a dominant negative rad51 allele should not be recovered due to this proliferation defect.

88 Altered alleles of mammalian rad51 that were engineered to be dominant negative were expressed in mouse embryonic stem cells. Due to the severity of the null phenotype, these experiments were designed to measure the absence of transfected cells by statistically relevant numbers. The first experiment measured the transfection efficiencies of vectors that expressed altered mammalian rad51 as compared to a vector that expressed wild-type mammalian RAD51, or vector alone. The altered transgenes, rad51TR1-131 and rad51K-A134, contained a functional protein binding region and a nonfunctional RecA homology region. For rad51TR1-131, a C-terminal truncation was made in the first ATP-binding domain (FIG. 1). For rad51K-A134, the conserved Lysine in the first ATP-binding motif was changed to an Alanine (for review, see Donovan and Weaver, 1994). rad51K-A134 more strongly associated with full length MmRad51 than rad51 TR1-131 as measured using the yeast two-hybrid system with about 90% relative .beta.-galactosidase activity (FIG. 1). The altered and wild-type transgenes were cloned into a CMV expression vector with a neomycin phosphotransferase (neo) cassette (pcDNA3 from invitrogen). Transfected embryonic stem (ES) cells were selected in G418 and colonies were counted 9 days later. The altered transgenes generated 20-30% fewer G418.sup.r colonies as compared to colonies resulting after transfection with wild-type MmRAD51 or vector alone in three experiments. Variations of 20-30% in transfection frequencies are commonly observed and are consequently not determinative in and of themselves. However, this minimal reduction could also indicate that the

toxic product of the altered transgenes was produced in sufficient quantities to stop cell proliferation. However, if the transgene product was truly toxic, then why did 70-80% of the cells survive in selection media? The transgene may be silent while the neo gene is expressed. The transgene may be disrupted upon integration into the chromosome or by chromosomal positional effects. In addition, strong expression of the transgene may be required to observe a phenotype while only weak expression of neo may be required for positive selection. Another experiment was needed to circumvent these possible problems.

89 5.5. Targeting the Expression Vectors to the RPRT Locus

90 Another experiment was developed to compare the targeting frequencies of vectors that expressed altered mammalian rad51 with vectors that expressed wild-type mammalian RAD51 or MC1tk (Herpes Simplex Virus type 1 thymidine kinase). The transgenes were targeted to the hypoxanthine phosphoribosyltransferase locus, HPRT (Melton et al., 1984, Proc. Natl. Acad. Sci. USA 81:2147-2151). Targeting the transgenes to HPRT would decrease the likelihood of disruption upon integration and Southern analysis could also be used to verify the integrity of the integration event (FIG. 3). The transgenes would also be located to a favorable environment for expression since HPRT is a house keeping gene, and thus all of the transgenes would be affected to the same degree by chromatin positional effects. The transgenes were cloned into the bacterial plasmid of an insertion vector that targeted HPRT (IVH). There were 6.9 kb of HPRT sequences that contained a neo cassette in exon 3. Therefore, upon linearization using a unique site in the homology region (an engineered NotI site), both insertion and replacement events could be recovered.

91 The targeting vectors were linearized in the HPRT homology region and transfected into ES cells. Transfected cells were selected for by growth in medium containing G418, and targeted cells were selected in medium containing G418+6-thioguanine (TG). G418 resistant (G418.sup.r) colonies were counted to measure the transfection efficiency and TG.sup.r +G418.sup.r colonies were counted to measure the targeting frequency.

TABLE 2

Targeting frequencies

	No. of Exp.	total Exps.	frequency total G418.sup.r	frequency TG.sup.r + G418.sup.r	target relative to IVH-tk
IVH-tk A	2	4088	338	1/12	NA
IVH-51TA	1	636	34	1/19	-37%
IVH-51KA	2	2792	106	1/26	-54%
IVH-tk B	2	1200	124	1/10	NA
IVH-51TA	2	472	22	1/21	-52%
IVH-51KA	2	1504	62	1/24	-58%
IVH-tk C	2	6016	264	1/23	NA
IVH-51WT	2	4840	192	1/26	-12%
IVH-51TA	2	2584	70	1/37	-38%
IVH-51KA	2	3664	48	1/76	-70%
IVH-tk D	2	6744	414	1/16	NA
IVH-51KA	2	4848	136	1/37	-57%
IVH-tk E	2	2624	186	1/14	NA
IVH-51WT	2	1456	84	1/17	-18%
IVH-51TA	2	2208	96	1/23	-39%
IVH-51KA	2	1376	52	1/26	-46%
IVH-tk F	2	1664	156	1/11	NA
IVH-51WT	2	752	60	1/12	-8%
IVH-51TA	2	760	34	1/22	-50%
IVH-51KA	2	544	30	1/18	-39%

92 Table 2: Electroporation: 10 .mu.g of NotI cut DNA/10.sup.7 cells/ ml PBS, 575 V/cm and 500 .mu.F. Each experiment (exps. A--F) shows results from electroporations that were done on the same day with a common batch of ES cells under identical conditions to eliminate variability. NA, not applicable.

93 The targeting frequencies of vectors that contained altered rad51 alleles were compared to control vectors (Table 2). Vectors that contained altered rad51 alleles were IVH-51TR1-131 (contains rad51TRI-131) and IVH-51KA (contains rad51K-A134). Control vectors were IVH-51WT (contains wild-type MmRAD51), and IVH-tk (contains MC1tk). The relative targeting frequencies (TG.sup.r +G418.sup.r /G418.sup.r colonies) were determined using IVH-tk efficiency as 100%. The relative targeting frequencies were reduced by 13+/-3.6% for IVH-51WT (average of three experiments), 43+/-6.4% for IVH-51TR1-131 (average of 5 experiments) and 54+/-7.6% for IVH-51KA (average of six experiments).

94 Southern analysis was performed on TG.sup.r +G418.sup.r clones to verify targeting and to identify the different targeting patterns (FIG. 3). Several types of recombination patterns were possible. A vector insertion event would integrate the entire vector to form a duplication of HPRT homology (Hasty et al., 1992, Molec. and Cell. Biol. 12:2464-2474). The vector may integrate on the 5' long arm or the 3' short arm (rarely observed). These integration patterns were combined since both integrate the transgene in between the duplication. A gene replacement event would introduce the neo but not the transgene and thus, provided a control. Modified events, that were not predicted by either pattern could also occur, and an intact transgene may or may not be introduced.

95 Comparison of the targeting patterns for the four vectors indicated that the transgene product was toxic for both rad51TR1-131 and rad51K-A134. The relative percentage of clones targeted with IVH-51TR1-131 and IVH-51KA that contained the transgene (vector insertion) decreased, and the relative percentage of targeted clones that did not contain the transgene (gene replacement) increased relative to controls. For both IVH-tk and IVH-51WT, targeting usually occurred by vector insertion (75% and 80%, respectively), rarely by gene replacement (14% and 17%, respectively), or more rarely by a modified event (6% and 8%, respectively). However, for IVH-51TR1-131 and IVH-51KA the relative frequency of targeted events that occurred by vector insertion decreased (68% and 45%, respectively), and gene replacement events increased (27% and 41%, respectively). The relative frequency of modified events also increased for clones targeted with IVH-51KA (14%). Therefore, the altered transgenes rarely integrated into the target locus as compared to the controls.

96 5.6. A High Percentage of Transfected Clones did not Express the Transgene

97 A statistically significant reduction in targeting frequency was observed using vectors that contained the altered rad51 alleles as compared to the wild-type allele or MC1tk. In addition, altered transgenes were introduced into HPRT for a lower percentage of the targeted clones as compared to the controls. However, targeted clones were generated that appeared to incorporate the altered transgenes intact. There are several possibilities for survival: 1) A small mutation may have been generated in the transgene; 2) The chromatin structure of the transgene may have been altered during the targeting event to silence the transgene (or vice- versa); 3) Position effect variegation may inhibit transcription of the transgene, but not neo.

98 Expression of MC1tk was tested in clones targeted with IVH-tk to determine the fraction of clones that do not express the transgene. Sixty-two TG.sup.r +G418.sup.r clones were grown in replica plates, one without FIAU and one with FIAU, to distinguish clones that lost or maintained HSV-1 thymidine kinase activity. A large percentage of clones (42%) survived in FIAU demonstrating that the IVH-51TR1-131 and IVH-51KA targeting frequencies were reduced to background levels. Therefore, all of the cells targeted with either

IVH-51TR1-131 and IVH-51KA that express the transgene were probably not recovered.

99 5.7. Conditional Expression of Amino Acids 1-43 of Mammalian Rad51 in ES cells Increases Sensitivity to .gamma.-radiation

100 It was demonstrated that the rad51.sup.M1 mutation increases sensitivity to .gamma.-radiation in early E3.5 day embryos and that dominant negative transgenes decrease proliferation of ES cells. Now, an expression vector that codes for amino acids 1-43 of Rad51 and is conditionally regulated by Doxycycline ("Dox") was introduced into the Hprt locus of ES cells. The expression vector is turned off by 5 ng/ml Dox. A vector that expresses the inducible tetR gene was transfected into the ES cells with the Rad51 1-43 expression vector in the presence of 5 ng/ml Dox. Ten clones were analyzed for sensitivity to .gamma.-radiation when grown in media with or with out Dox. Cells grown with Dox (transgene turned off) were more resistant to .gamma.-radiation than cells grown without it, demonstrating that amino acids 1-43 of Rad51 sensitizes cells to radiation (FIG. 4a). Since ES cells are immortal and transformed, these data demonstrate that disrupting the Rad51 pathway will serve as a therapeutic for cancer. Therefore, expression of dominant negative transgenes that code for any protein that will disrupt mammalian Rad51 function could serve as a therapeutic for cancer and should not be limited to just the first 43 amino acids of Rad51.

101 5.8. Application of a Peptide that Inhibits Cell Proliferation by Disrupting Mammalian Rad51

102 It was demonstrated that mammalian Rad51 interacts with mammalian Brca2. A peptide of the amino acid sequence ROIKIWFONRRMKWKKFLSRLPLPSPVSPICTFVSPAAQKAFQPPRS was synthesized. This peptide contains the region of Brca2 that interacts with Rad51, amino acids 3196-3226 (not underlined), SEQ ID NO. 3. This peptide also contains 16 amino acids derived from the Drosophila Antennapedia protein (underlined) SEQ ID NO:4that translocates through biological membranes. This peptide was added to media after p53.sup.-/- fibroblasts were plated at low concentration (100 cells/ 6 cm plate). Colonies were counted based on size as determined by the number of cells. The peptide caused a great reduction of colonies composed of 265 or greater cells (FIG. 4b). Thus, the peptide had a profoundly negative effect on cellular proliferation. The 16 amino acids derived from Antennapedia had no effect on the number of colonies at any size; therefore, the inhibitory affect was due to the Brca2 sequences. Since p53.sup.-/- cells are highly proliferative and commonly found in cancer, these data demonstrate that disrupting the Rad51 pathway will serve as a therapeutic for cancer. Therefore, any peptide that interacts with mammalian Rad.sup.51 may inhibit proliferation of cells in tissue culture and could be used to inhibit the growth of cancer.

103 5.9. Application of Molecules that Disrupt Mammalian Rad51 and/or Rad52 Function for Cancer Therapeutics

104 The rad51.sup.M1 mutation reduces proliferation and promotes cellular senescence, even in a p53 mutant background. In addition, rad51 dominant negative alleles also display this phenotype by presumably forming nonproductive protein associations with Rad51 and other proteins like Rad52, M96 and Brca2. Therefore, it is likely that the disruption of mammalian Rad51, mammalian Rad52 (or any protein in the DSB repair pathway mediated by these proteins) will reduce cell proliferation or induce cell death, and thus be suitable as a cancer therapeutic. In addition, the disruption of any protein--protein association important for mammalian Rad51 function or mammalian Rad52 function will also reduce cell proliferation or induce cell death, and thus be suitable as a cancer therapeutic.

105 Additionally, dominant negative alleles of rad51 may be used to express cancer therapeutics that reduce cell proliferation or induce cell death. An expression vector that codes for a dominant negative rad51 allele may be introduced into cancer cells, or an mRNA that codes for a dominant negative rad51 allele may be

introduced into cancer cells, or a dominant negative Rad51 protein may be introduced into cancer cells. Several examples of such dominant negative rad51 alleles are presently disclosed. Of these alleles, the protein encoded by rad51K-A131 appears to have the strongest self-association, and proved toxic to proliferating cells. In fact, any rad51 allele that rendered the RecA homology region nonfunctional but preserved the N-terminal protein association region should reduce cell proliferation or induce cell death and could thus be

106 used as a cancer therapeutic.

107 In addition to subtle alterations in the RecA core homology region of mammalian Rad51, C-terminal truncations in mammalian rad51 may also be used to reduce cell proliferation and/or induce cell death. rad51TR1-131 demonstrated a toxic effect on cells even though it had a relatively weak interaction with MmRad51 which suggested that the phenotype might be caused by nonfunctional self-associations, or nonfunctional associations with other proteins such as Rad52, M96 and Brca2. rad51TR1-43 had a strong interaction with MmRad51 and may be more effective as a cancer therapeutic than rad51TR1-131. In fact, any C-terminal truncation that preserves the protein interacting region of Rad51 may be used as a dominant negative allele for cancer therapy. Additionally, fusion of the N terminal domain of mammalian Rad51 to the 16 or 60 amino acids of the 3rd helix of the antennapedia protein may promote entry into the nucleus (Derossi et al., 1994, J. Bio. Chem. 269:10444-10450).

108 Mammalian Rad51 interacts with other proteins besides itself, and disruption of these interactions could be used to reduce cell proliferation or induce cell death. Other proteins interacting with mammalian Rad51 include but are not limited to mammalian Rad52, Brca2 and M96.

109 The identification of other interacting proteins will further elucidate the pathway and present greater opportunities to disrupt this pathway for the purpose of hindering cell proliferation. Since mammalian Rad52 associates with mammalian Rad51 and other proteins (Park et al., 1996; Shen et al., 1996), dominant alleles of mammalian Rad52 may also hinder cell proliferation or induce cell death. Such alleles could also be used for cancer therapeutics. In fact, dominant alleles of any protein that associates with mammalian Rad51, Rad52 or any other protein in these pathways, may be expected to hinder cell proliferation or induce cell death. Thus, all of the above molecules collectively define a new class of therapeutic agents for the treatment of proliferative disorders, viral infection (especially HIV infection), and cancer.

110 EQUIVALENTS

111 The foregoing specification is considered to be sufficient to enable one skilled in the art to broadly practice the invention. Indeed, various modifications of the above-described methods for carrying out the invention, which are obvious to those skilled in the field of microbiology, biochemistry, organic chemistry, medicine or related fields, are intended to be within the scope of the following claims. All patents, patents applications, and publications cited herein are incorporated by reference.

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#40
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            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
  - -      (ii) MOLECULE TYPE: peptide
  - -      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
  - - Phe Leu Ser Arg Leu Pro Leu Pro Ser Pro Va - #l Ser Pro Ile Cys Thr
  1               5  - #                10  - #                15
  - - Phe Val Ser Pro Ala Ala Gln Lys Ala Phe Gl - #n Pro Pro Arg Ser
            20       - #           25       - #           30
  - -  - - (2) INFORMATION FOR SEQ ID NO:4:
  - -       (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 16 amino - #acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
  - -      (ii) MOLECULE TYPE: peptide
  - -      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
  - - Arg Gln Ile Lys Ile Trp Phe Gln Asn Arg Ar - #g Met Lys Trp Lys Lys
  1               5  - #                10  - #                15
```

CLAIMS:

What is claimed is:

1. The truncated Rad51 product encoded by rad51TR1-131.

2. The altered Rad51 product encoded by rad51K-A134.

3. A polypeptide encoded by a polynucleotide which hybridizes under stringent conditions to a second polynucleotide that is complementary to a nucleotide sequence that encodes the amino acid sequence of SEQ ID NO:1 from residue 1 through 131, said polypeptide inhibits cell proliferation.

4. The polypeptide of claim 3 wherein the nucleotide sequence encodes the amino acid sequence of SEQ ID NO:1 from residue 1 through 43, said polypeptide inhibits cell proliferation.

5. A polypeptide encoded by a polynucleotide which hybridizes under stringent conditions to a second polynucleotide that is complementary to a nucleotide sequence that encodes the amino acid of SEQ ID NO:3, said polypeptide inhibits cell proliferation.

6. A polypeptide consisting essentially of the amino acid sequence of SEQ ID NO:1 from residue 1 to 131.

7. A polypeptide consisting essentially of the amino acid sequence of SEQ ID NO:1 from residue 1 to 43.

8. A polypeptide consisting essentially of the amino acid sequence of SEQ ID NO:3.

9. A polynucleotide that encodes the amino acid sequence of SEQ ID NO:1 from residue 1 to 131.

10. A polynucleotide that encodes the amino acid sequence of SEQ ID NO:1 from residue 1 to 43.

11. A polynucleotide that encodes the amino acid sequence of SEQ ID NO:3.

12. A method of screening for compounds that hinder cell proliferation or that promote programmed cell death, comprising:

a) assaying for microsatellite formation in cells;

b) assaying for chromosome loss in cells; or

c) assaying for the disruption of strand exchange in an in vitro assay.

WEST

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L2: Entry 2 of 4 File: USPT Mar 14, 2000

US-PAT-NO: 6037125
DOCUMENT-IDENTIFIER: US 6037125 A

TITLE: Disruption of the mammalian RAD51 protein and disruption of proteins that associate with mammalian RAD51 for hindering cell proliferation and/or viability of proliferating cells

DATE-ISSUED: March 14, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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APPL-NO: 08/ 758280 [PALM]
DATE FILED: November 5, 1996

INT-CL: [07] C12 Q 1/68

US-CL-ISSUED: 435/6
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FIELD-OF-SEARCH: 435/6, 435/172.3, 435/441

PRIOR-ART-DISCLOSED:

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ART-UNIT: 185

PRIMARY-EXAMINER: Ketter; James

ABSTRACT:

When a mutation, designated rad51.sup.M1, was generated in the mouse MmRAD51 gene, mutant embryos died shortly after implantation. rad51.sup.M1 cells exhibited hypersensitivity to ionizing radiation, reduced proliferation, programmed cell death and chromosome loss. The disruption of MmRad51 rotein-protein interactions stopped cell proliferation and/or reduced cell viability. Several proteins that interact with MmRad51 have been identified including, for example Brca2 and M96. Additionally, Rad51 self-associates via the N-terminal region. When a single residue was changed from a conserved lysine to an alanine, the alteration proved toxic to cells. Moreover, a rad51 allele that lacked the RecA homology region was also deleterious to cells. In view of the above, it is clear that inhibiting MmRad51 function or the function of any molecule that associates with MmRad51, or any molecule in the Rad51 or Rad52 pathways, hinders cell proliferation and/or viability. Accordingly, molecules capable of blocking these critical DNA repair pathways may be effective as therapeutics for inhibiting cell proliferation.

15 Claims, 3 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 2

BRIEF SUMMARY:

1 1.0. FIELD OF THE INVENTION

2 The present invention relates to molecules that disrupt mammalian Rad51 or Rad52 function, or disrupt the function of other molecules that are involved in the Rad51 or Rad52 pathways. Such molecules are useful as a means to hinder cell proliferation or to promote programmed cell death, and define a novel class of therapeutic agents for use in the treatment of proliferative disorders such as autoimmune disease and cancer.

3 2.0. BACKGROUND OF THE INVENTION

4 DNA repair and recombination are required by organisms to prevent the accumulation of mutations and to maintain the integrity of genetic information. Damaged genetic material may result in cell cycle arrest, programmed cell death, chromosome loss or cell senescence. Alternatively, compromised genetic information may result in dysregulation of the cell cycle ultimately leading to increased cellular growth and tumor formation.

5 The repair of double-strand breaks (DSB) in DNA is an essential cellular process. DSB repair may occur during general cellular functions such as DNA repair (Friedberg et al., 1995, DNA Repair and Mutagenesis. American Society for Microbiology, Washington, D.C.). In bacteria and yeast cells, DSB are predominately repaired by a homologous recombination pathway (Krasin and Hutchinson, 1977, J. Mol. Biol. 116:81-98; Mortimer, 1958, Radiat. Res. 9:312-16. In the budding yeast Saccharomyces cerevisiae the RAD52 epistasis group (Rad50 to Rad57, Mre11 and Xrs2) was identified in cells sensitive to ionizing radiation (reviewed in Friedberg, 1995; Petes et al., 1991, Recombination in yeast., p. 407-521. In J. R. P. J. R. Broach, and E. W. Jones (ed.), The Molecular and Cellular Biology of the Yeast Saccharomyces. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). Later, some of the members of this group were shown to be important for recombinational repair (e.g., Rad51, Rad52, Rad54, Rad55, Rad57 (Malkova et al., 1996, Proc. Natl. Acad. Sci. USA 93:7131-36, Sugawara et al., 1995, Nature 373:84-86).

6 Among the members of the RAD52 epistasis group, ScRad51 is particularly interesting because it shares similarity with the Escherichia coli recombination protein, RecA. ScRad51 and RecA polymerize on double-stranded and single-stranded DNA (dsDNA, ssDNA) to produce a helical filament, and both enzymes catalyze an ATP-dependent strand exchange between homologous DNA molecules (Ogawa et al., 1993, Science 259:1896-99; Sung, 1994, Science 265:1241-4364; Sung and Robberson, 1995, Cell 82:453-61). ScRad51 and RecA share 30% homology over a span of about 220 amino acids, and each protein

contains two conserved ATP binding motifs (Aboussekhra et al., 1992, Mol. and Cell. Biol. 12:3224-34; Basile et al., 1992, Mol. Cell. Biol. 12:3235-46; Sugawara et al., 1995, Nature 373:84-86).

7 ScRad51 repairs DSB by homologous recombination. DSB accumulate at recombination hot spots during meiosis in cells that lack ScRad51 (Sugawara, 1995), and ScRad51 localizes to meiotic nuclei (Bishop, 1994, Cell 79:1081-92) and promotes meiotic chromosome synapsis (Rockmill et al., 1995, Genes & Develop. 9:2684-95). Accordingly, it is thought that ScRad51 mediates meiotic recombination by binding to single-strands generated at DSB which are in strand pairing and exchange during meiosis (Sung and Robberson, 1995, Cell 82:453-61).

8 A variety of direct and indirect protein-protein interactions are essential for RecA and ScRad51 function. The crystal structure of RecA suggests that a portion of the N-terminal region is involved in polymer formation (Story et al., 1993, Science 259:1892-96; Story et al., 1992, Nature 355:318-324) which was supported by genetic analysis that showed C-terminal truncations dominantly interfered with DNA repair in wild-type bacteria (Horii et al., 1992, J. Mol. Biol. 223:104-114; Tateishi et al., 1992, J. Mol. Biol. 223:115-129; Yarranton et al., 1982, Mol. Gen. Genet. 185:99-104). A similar self-association region occurs in the N-terminal region of ScRad51 and is essential for DNA repair (Donovan et al., 1994, Genes & Develop. 8:2552-2562; Shinohara et al., 1992, Cell 69:457-70). ScRad51 also associates with Rad52 and Rad55 (Hays et al., 1995, Proc. Natl. Acad. Sci USA 92:6925-6929; Johnson and Symington, 1995, Molec. Cell. Biol. 15(9):4843-4850; Milne and Weaver, 1993, Genes & Develop. 7:1755-1765) as well as other proteins. Other protein interactions may be inferred because a rad51.DELTA. rad52.DELTA. strain of S. cerevisiae was only partially complemented by Rad51 and Rad52 from Kluyveromyeces lactis (Donovan et al., 1994, Genes & Develop. 8:2552-2562), and because ScRad51 colocalized with Dmc1 to the synaptonemal complex (Bishop, 1994, Cell 79:1081-92). These data suggest that a large protein complex is necessary for recombinational repair and that disruption of any of the proteins in this complex hinders the repair of DSB.

9 RecA/ScRad51 homologues have been discovered in a wide range of organisms including the fission yeast Schizosaccharomyces pombe (Jang et al., 1994, Gene 142:207-11; Muris et al., 1993, Nuc. Acids Res. 21:4586-91; Shinohara et al., 1993, Nature Genet. 4:239-4358), lilies (Terasawa et al., 1995, Genes & Develop. 9:925-34), chickens (Bezzubova et al., 1993, Nucl. Acids Res. 21:1577-80), mice (Morita et al., 1993, Proc. Natl. Acad. Sci USA 90:6577-80; Shinohara et al. 1993, Nature Genet. 4:239-43) and humans (Shinohara et al. 1993; Yoshimura et al., 1993, Nucl. Acids Res. 21:1665), and appear to be involved in DNA repair and recombination based on the following evidence: 1) Conserved RecA homology--MmRad51 is 83% homologous, 69% identical to ScRad51, and 51% homologous, 28% identical to RecA. Shared homology between mammalian and yeast Rad51 suggest conserved function due to the remarkable similarity between other mammalian and yeast DNA repair pathways (reviewed in Cleaver, 1994, Cell 76:1-4); 2) Expression pattern--MmRAD51 is highly expressed in tissues involved in meiotic recombination such as testes (Morita et al., 1993, Proc. Natl. Acad. Sci USA 90:6577-80) and ovaries (Shinohara et al., 1993, Nature Genet. 4:239-43). Additionally, expression of the S. pombe MmRad51 homologue SpRAD51 increased after cells were treated with methyl methanesulfonate which provides further evidence of a DNA repair function (Jang et al., 1994, Gene 142:207-11); 3) Protein cellular localization--Mouse, chicken, and lily Rad51 localizes at discrete foci on meiotic chromosomes at varying concentrations during prophase 1, possibly on the lateral elements and recombination nodules, which suggests a role in the repair of DSB during meiotic recombination (Ashley et al., 1995, Chromosoma 104:19-28; Haaf et al., 1995, Proc. Natl. Acad. Sci. USA 92:2298-2302; Terasawa et al., 1995). Moreover, increasing concentrations of human Rad51, HsRad51, localize to the nucleus after exposure to DNA damaging agents which also suggests a repair function (Terasawa et al., 1995); 4) Filament formation on DNA--HsRad51 bind to ssDNA which demonstrates a potential for strand exchange (Benson et al., 1994, EMBO 13:5764-71); 5) Mouse cells with a rad51 mutation, designated rad.51.sup.M1, displayed features that are known to be characteristic of

unrepaired DSB in yeast cells (Lim and Hasty, 1996, In press) which include reduced proliferation, hypersensitivity to .gamma.-radiation, chromosome loss and programmed cell death.

10 The function of MmRad51 is not completely understood; however, it is thought that, like ScRad51, it has, inter alia, a recombinational repair function. The recombinational repair pathway appears to be at least partially conserved between yeast and mammals. Mammalian homologues have been found for the members of the yeast Rad52 epistasis group (Rad51, Rad52), and to other yeast proteins (Dmc1) implicated in recombinational repair (Bendixen et al., 1994, Genomics 23:300-3035, Habu et al. 1996, Nucleic Acids Res. 24:470-7719; Morita et al., 1993, Proc. Natl. Acad. Sci USA 90:6577-80; Shen et al., 1995, Genomics 25:199-206; Shinchara et al., 1993). Expression pattern analysis supported the hypothesis that these homologues performed the same functions in yeast and mammals. MmRAD51 was highly expressed in tissues with cells involved in meiotic recombination, testis and ovary, and rapid cell division, intestine, embryo, and thymus (Morita et al., 1993; Shinohara et al., 1993). A role during meiotic recombination was further suggested because MmRAD51 was highly enriched in the synaptonemal complex in pachytene spermatocytes (Ashley et al., 1995; Haaf et al., 1995).

11 The most compelling evidence that MmRad51 and ScRad51 function is conserved comes from analysis of rad51 mutant cells, the mutation was designated rad51.sup.M1 (Lim and Hasty, 1996).

12 3.0. SUMMARY OF THE INVENTION

13 An object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting mammalian Rad51 function.

14 An additional object is to hinder cell proliferation or reduce cell viability by disrupting mammalian Rad52 function.

15 Another object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting proteins that associate with mammalian Rad51.

16 Another object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting proteins that associate with mammalian Rad52.

17 Another object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting any proteins involved in the mammalian Rad51 or mammalian Rad52 pathways.

18 Another object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting mammalian Rad51 protein interactions.

19 Another object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting mammalian Rad52 protein interactions.

20 Another object of the present invention is to hinder cell proliferation or reduce cell viability by disrupting protein-protein interactions that are involved in the mammalian Rad51 or mammalian Rad52 pathways.

21 Yet another embodiment of the present invention involves methods of identifying compounds that are capable of inhibiting the binding or function of any protein involved in the Rad51 pathway, and, in particular, compounds capable of binding or inhibiting the function of Rad51 protein. Accordingly, an additional embodiment of the present invention involves methods of screening for compounds that disrupt double-stranded break repair by assaying for microsatellite formation in cells; assaying for chromosome loss in cells; assaying for the disruption of strand exchange in an in vitro assay; assaying for decreased cell proliferation; assaying for premature replicative cellular senescence; and assaying for increased cell death.

DRAWING DESCRIPTION:

4.0. BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. mRNA structure of MmRAD51. The predicted amino acids are numbered according to Shinohara et al., 1993. The shaded box represents the recA homology region. The open boxes represent regions that are not conserved across species. The thick vertical lines represent the ATP binding domains.

FIG. 2. MmRad51 self-association as demonstrated by the yeast two-hybrid system. The self-association is restricted to the most N-terminal 43 amino acids. The shaded box is the RecA core homology region (Shinohara et al, 1993). The thick vertical lines represent the ATP-binding sites. The open boxes represent regions that are not conserved between species. The relative .beta.-galactosidase (.beta.-gal) activities are presented, right panel. Full length wild-type MmRad51 is considered to be 100%. E12 served as a negative control and had 1% relative activity.

FIG. 3. Targeting the transgenes to the hprt locus. The hprt sequences contain exons 2 and 3 (labeled boxes). Hprt homology of vector origin is a thick line, of chromosomal origin is a thin line. The bacterial plasmid is represented by a wavy line. Potential locations for crossovers are X's labeled 1 or 2. Two recombination events are possible: either gene replacement with crossovers at both 1 and 2 or vector insertion with crossovers at either 1 or 2. For vector insertion, only a crossover at 1 is shown.

DETAILED DESCRIPTION:

1 4.0. DETAILED DESCRIPTION OF THE INVENTION

2 As discussed above, one embodiment of the present invention is the expression of altered mammalian rad51 alleles that disrupt mammalian Rad51 function, mammalian Rad52 function, or the function of any other protein in the mammalian Rad51 or Rad52 pathways. Although the presently described invention has been specifically exemplified using a species exemplary of the order mammalia, given the relatively high level of interspecies sequence similarity (and functional similarity) observed in the Rad51 proteins, it is clear that the present invention may be broadly applied to other mammalian species, including humans, as well as nonmammalian animals such as birds, and fish.

3 In addition to mice, examples of mammalian species that may be used in the practice of the present invention include, but are not limited to: humans, non-human primates (such as chimpanzees), pigs, rats (or other rodents), rabbits, cattle, goats, sheep, and guinea pigs.

4 Reduced proliferation, hypersensitivity to .gamma.-radiation, chromosome loss, and cell death have all been associated with rad51.sup.M1 cells. These characteristics are similar to those seen in yeast cells deficient for recombinational repair either due to sequence divergence, or due to a mutation in rad51 or rad52 (Malkova et al., 1996, Proc. Natl. Acad. Sci. USA 93:7131-36; Resnick et al., 1989, Proc. Natl. Acad. Sci. USA 86:2276-80; Tsuzuki et al., 1996, Proc. Natl. Acad. Sci USA 93:6236-40). Even though these data suggest MmRad51 functions during recombinational repair it is also possible that the severe phenotype observed in rad51.sup.M1 cells was due to disruption of another process.

5 For the purposes of the present application the term ionizing radiation shall mean all forms of radiation, including but not limited to .alpha., .beta., and .gamma. radiation and U.V. light, which are capable of directly or indirectly damaging the genetic material of a cell or virus. The term irradiation shall mean the exposure of a sample of interest to ionizing radiation, and the term radiosensitive shall refer to cells or individuals which display unusually

adverse consequences after receiving moderate, or medically acceptable (i.e., nonlethal diagnostic or therapeutic doses), exposure to ionizing irradiation.

6 There is evidence that RecA homologues perform multiple functions, and that all functions are not performed by every homolog. For instance, of the two RecA homologues found in Myxococcus xanthus, only one is essential; however, both complement UV sensitivity in an E. coli recA strain (Norioka et al., 1995, J. Bacteriol. 177:4179-82). Also, two RecA homologues found in yeast, ScRad51 and Dmc1, are essential for meiotic recombination, but only ScRad51 is essential for mitotic recombination (Bishop, 1994, Rockmill et al. 1995, Genes & Develop. 9:2684-95). In mammals, a Dmc1 homologue has been isolated which suggests that, like yeast, mammalian RecA homologues also perform diverse and unique functions in mammalian cells (Habu et al., 1996).

7 MmRad51 may perform a novel role in DNA replication, repair, or chromosomal disjunction. MmRAD51 expression is restricted during the cell cycle to late G.sub.1 /S/G.sub.2 and MmRAD51 expression was activated by mitogens that induced T and B cell proliferation suggesting a role in replication and repair (Yamamoto et al., 1996, 251:1-12). MmRad51 may take part in disjunction because it localizes to the kinetochores of diakinesis, and metaphase 1 chromosomes (Ashley et al., 1995).

8 The exact function or functions performed by MmRad51 are unimportant with regard to developing anti-proliferative drugs and cancer therapeutics as long as the disruption of the MmRad51 function provides a benefit to the patient. For the purposes of the present invention, it is assumed that the function of Rad51 is the repair of DSB; however, it is likely that Rad51 performs additional functions in the cell. However, it is important to note that at least some aspect of MmRad51 function is essential for cell proliferation and/or viability, and that molecules capable of disrupting MmRad51 function thus hinder cell proliferation or reduce cell viability. As such, any molecule that disrupts the MmRad51 pathway should prove useful for cancer therapy (for example). Furthermore, disruption of any protein-protein interaction that involves either MmRad51 or any other molecule in the MmRad51 pathway should also prove useful for cancer therapy.

9 Protein-protein interactions are critical for recombinational repair in yeast cells, including interactions that involve ScRad51 and ScRad52 (Donovan et al., 1994; Milne et al., 1993). In addition, the human Rad51 and Rad52 proteins were shown to associate like their yeast homologues (Shen et al., 1996, J. Biol. Chem. 271:148-152).

10 To isolate proteins that associate with MmRad51, a yeast two-hybrid screen was performed with MmRad51 as the "bait" and a T cell library and an embryonic cell library as the "prey". Among other proteins identified using this screen, MmRad51 and Brca2 were isolated, and the interactions identified using this screen may prove critical for in vivo function. Additional biochemical binding assays that may prove useful for identifying compounds that are able to associate with MmRad51 (or any other target protein) are well known in the art including, but not limited to: equilibrium or membrane flow dialysis, antibody binding assays, gel-shift assays, in vitro binding assays, filter binding assays, enzyme-linked immunoabsorbent assays (ELISA), western blots, co-immunoprecipitation, immunogold co-immunoprecipitation, coimmunolocalization, co-crystallization, fluorescence energy transfer, competition binding assays, chemical crosslinking, and affinity purification. In addition, genetic analysis may be used to identify accessory proteins that interact with MmRad51 or are peripherally involved in MmRad51 function. Where the MmRad51 accessory protein is essential to MmRad51 function, mutation in the genes encoding these proteins should typically result in phenotypes similar to those associated with MmRad51 mutations. Similarly, where the MmRad51 accessory proteins function to inhibit or retard MmRad51 activity, mutations in the genes encoding these factors shall generally mimic antagonist phenotypes.

11 The MmRad51 self-association was investigated further. Deletion analysis revealed that the MmRad51 self-association occurred in the N-terminal region

which further demonstrated conservation of function with ScRad51 and RecA since both were shown to self-associate via the N-terminal region of the protein (Donovan et al., 1994; Horii, 1992; Story et al., 1992, 1993; Tateishi et al., 1992; Yarranton and Sedgwick, 1982).

12 Given the critical importance of mammalian Rad51 function, any disruption of the mammalian Rad51 or Rad52 complexes, or any member in their pathway will necessarily hinder cell proliferation or viability. When the Rad51 and Rad52 pathways were disrupted by introducing altered mouse rad51 into mouse cells, nonproductive protein-protein associations resulted. The altered forms of mouse rad51 were generated by disrupting a conserved nucleotide binding motif while preserving the protein association domain. The expression of these transgenes resulted in cellular toxicity. Presumably, the resulting nonproductive protein associations were responsible for the drastically reduced viability of these cells. In view of this result, it is clear one may reduce cell proliferation by disrupting mammalian Rad51 function, or the function of any protein in this repair pathway by hindering protein association by using defective proteins or other means such as small molecules.

13 Given that the Rad51 proteins are known to self-associate, the Rad51 protein sequence provides a template for the identification and genesis of peptides or factors that disrupt Rad51 function or activity. Accordingly, an additional embodiment of the present invention are peptides or polypeptides that correspond at least five contiguous amino acids of the mammalian Rad51 amino acid sequence (SEQ ID NO. 1), or the human Rad51 amino acid sequence (SEQ ID NO. 2) that retain the property of being capable of binding a mammalian Rad51 and/or inhibiting Rad51 function (as detected using a suitable biochemical, genetic, or cellular assay).

14 Additionally, the blocking of normal Rad51 function may induce programmed cell death. Thus, one aspect of the present invention are a novel class of therapeutic agents, factors, or compounds that have been engineered, or are otherwise capable of disrupting the essential processes that are mediated by, or associated with, normal Rad51 or Rad52 activity. Accordingly, it is contemplated that this novel class of therapeutics agents may be used to treat diseases including, but not limited to, autoimmune disorders and diseases, inflammation, cancer, graft rejection, and any of a variety of proliferative or hyperproliferative disorders.

15 Typical examples of therapeutic agents based on the above presently described molecules include, but are not limited to, defective (either engineered or naturally occurring) forms of the proteins that associate with the protein complexes, inhibitory fragments of the proteins, wild type and altered genes that code for proteins that disrupt mammalian Rad51 function, small organic molecules, antisense nucleic acid sequences, oligonucleotides that inhibit expression or activity via a triplex mechanism, peptides, aptameric oligonucleotides, and the like.

16 More particularly, examples of engineered proteins may include, but are not limited to, proteins that comprise inactivating mutations in conserved active sites (e.g., ATP binding motifs, DNA or protein binding domains, catalytic sites, etc.), fusion proteins that comprise at least one inhibitory domain, and the like.

17 The above agents may be obtained from a wide variety of sources. For example, standard methods of organic synthesis may be used to generate small organic molecules that mimic the desired regions of the target DNA repair proteins. In addition, combinatorial libraries comprising a vast number of compounds (organic, peptide, or nucleic acid, reviewed in Gallop et al. 1994, J. Med. Chem. 37(9):1233-1251; Gordon et al., 1994, J. Med. Chem. 37(10):1385-1401; and U.S. Pat. No. 5,424,186 all of which are herein incorporated by reference) may be screened for the ability to bind and inhibit the activity of proteins involved in DSB repair or any other potential mammalian Rad51 function.

18 In particular, inhibitory peptides should prove very useful. Such compounds may

include, but are not limited to, peptides such as, for example, soluble peptides, including but not limited to members of random peptide libraries; (see, e.g., Lam et al., 1991, Nature 354:82-84; Houghten et al., 1991, Nature 354:84-86), and combinatorial chemistry-derived molecular library made of D- and/or L-configuration amino acids, phosphopeptides (including, but not limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang et al., 1993, Cell 72:767-778).

19 Given that they will serve as templates for the rational design of agents for disrupting DSB repair activity in the cell, it would be advantageous to purify each of the individual proteins that are directly or indirectly involved in DSB repair of any other potential mammalian Rad51 function. The various proteins involved in the DSB repair pathways may be purified using any of a number of variations of well established biochemical, and molecular biology techniques. Such techniques are well known to those of ordinary skill in the biochemical arts and have been extensively described in references such as Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology, Volume 152, Academic Press, San Diego, Calif. (1987; Molecular Cloning: A Laboratory Manual, 2d ed., Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989); Current Protocols in Molecular Biology, John Wiley & Sons, all Vols., 1989, and periodic updates thereof); New Protein Techniques: Methods in Molecular Biology, Walker, J. M., ed., Humana Press, Clifton, N.J., 1988; and Protein Purification: Principles and Practice, 3rd. Ed., Scopes, R. K., Springer-Verlag, New York, N.Y., 1987. In general, techniques including, but not limited to, ammonium sulfate precipitation; centrifugation, ion exchange, gel filtration, and reverse-phase chromatography (and the HPLC or FPLC forms thereof) may be used to purify the various proteins of the DSB repair complex.

20 Additionally, purified preparations of the presently described DNA repair proteins, associated proteins, or fragments thereof, may be used to generate antisera specific for a given agent. Accordingly, additional embodiments of the present invention include polyclonal and monoclonal antibodies that recognize epitopes of the presently described DNA repair complex proteins. The factors used to induce the antibodies of interest need not be biological active; however, the factors should induce immunological activity in the animal used to generate the antibodies.

21 Given that similar methodologies may be applied to the generation of antibodies to the various factors, for purposes of convenience, only the Rad51 factor antibodies will be discussed further.

22 Polypeptides for use in the induction of Rad51-specific antibodies may have an amino acid sequence consisting of at least three amino acids, and preferably at least 10 amino acids, that mimic a portion of the amino acid sequence of Rad51, and may contain the entire amino acid sequence of naturally occurring Rad51 or a Rad51-derivative.

23 Anti-Rad51 antibodies are expected to have a variety of medically useful applications, several of which are described generally below. More detailed and specific descriptions of various uses for anti-Rad51 antibodies are provided in the sections and subsections which follow. Briefly, anti-Rad51 antibodies may be used for the detection and quantification of Rad51 polypeptide expression in cultured cells, tissue samples, and in vivo. Such immunological detection of Rad51 may be used, for example, to identify, monitor, and assist in the prognosis of neoplasms that have been treated with factors that inhibit DSB repair. Additionally, monoclonal antibodies recognizing epitopes from different parts of the Rad51 structure may be used to detect and/or distinguish between native Rad51 and various subcomponent and/or mutant forms of the molecule. Additionally, anti-Rad51 monoclonal antibodies may be used to test preparations of agents or factors that mimic segments of Rad51, or are designed to impair protein association with Rad51, or to competitively inhibit DNA binding. In addition to the various diagnostic and therapeutic utilities of anti-Rad51 antibodies, a number of industrial and research applications will be obvious to those skilled in the art, including, for example, the use of anti-Rad51 antibodies as affinity reagents for the isolation of Rad51-associated

polypeptides, and as immunological probes for elucidating the biosynthesis, metabolism and biological functions of Rad51. Rad51 antibodies may also be used to purify Rad51 or Rad51-associated factors by affinity chromatography.

24 Once purified, the proteins of interest may be partially sequenced, and these data may be used to design degenerate oligonucleotide probes for use in cloning the genes encoding the various proteins that are associated with DSB repair. Alternatively, any of a variety of public or private sequence data bases may be searched for nucleic acid or peptide sequences that share homology with genes and proteins associated with Rad51-mediated DSB repair. Once a similar sequence is identified, peptides may be produced and screened for inhibitory activity. Where a nucleic acid library is involved, one could synthesize a probe corresponding to the nucleic acid sequence of interest, and use the probe to clone a full-length version of the corresponding gene (if necessary). Accordingly, an additional embodiment of the presently claimed invention are nucleic acid sequences that are capable of hybridizing to sequences encoding the proteins that are associated with DSB repair under stringent conditions. For the purposes of the present invention, the term "stringent conditions" generally refers to hybridization conditions that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50.degree. C.; or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42.degree. C.; or (3) employ 50% formamide, 5.times.SSC (0.75 M NaCl, 0.075 M Sodium pyrophosphate, 5.times.Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42.degree. C., with washes at 42.degree. C. in 0.2.times.SSC and 0.1% SDS. The above examples of hybridization conditions are merely provided for purposes of exemplification and not limitation. A more thorough treatise of the such routine molecular biology techniques may be found in Sambrook et al., Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., Vols. 1-3: (1989), and periodic updates thereof, herein incorporated by reference.

25 Once isolated, the genes encoding the proteins involved in DSB repair may be recombinantly expressed using standard vectors and hosts. Examples of vectors that may be used to express proteins of interest are provided in Sambrook et al., Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., Vols. 1-3: (1989). In particular, eucaryotic viruses may be used as vectors to transduce any of a wide variety of plant and animal cells to over express the desired proteins. Examples of such viruses include, but are not limited to, adenovirus, papilloma virus, herpes virus, adeno-associated virus, rabies virus, bacculo virus, retrovirus, plant viruses, and the like (See generally, Sambrook et al., Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., Vol. 3:16.1-16.89 (1989); U.S. Pat. No. 5,316,931, issued May 31, 1994, herein incorporated by reference).

26 Preferably, agents that disrupt DSB repair shall be substantially specific for blocking the desired repair pathways. For the purposes of the present invention, the term substantially specific shall mean that a given agent is capable of being dosaged to provide the desired effect while not causing undue cellular toxicity.

27 One of ordinary skill will appreciate that, from a medical practitioner's or patient's perspective, virtually any alleviation or prevention of an undesirable symptom (e.g., symptoms related to disease, sensitivity to environmental or factors, normal aging, and the like) would be desirable. Thus, for the purposes of this Application, the terms "treatment", "therapeutic use", or "medicinal use" used herein shall refer to any and all uses of compositions comprising the claimed agents which remedy a disease state or symptoms, or otherwise prevent, hinder, retard, or reverse the progression of disease or other undesirable symptoms in any way whatsoever.

28 When used in the therapeutic treatment of disease, an appropriate dosage of presently described agents, or derivatives thereof, may be determined by any of

several well established methodologies. For instance, animal studies are commonly used to determine the maximal tolerable dose, or MTD, of bioactive agent per kilogram weight. In general, at least one of the animal species tested is mammalian. Those skilled in the art regularly extrapolate doses for efficacy and avoiding toxicity to other species, including human. Before human studies of efficacy are undertaken, Phase I clinical studies in normal subjects help establish safe doses.

29 Additionally, the bioactive agents may be complexed with a variety of well established compounds or structures that, for instance, enhance the stability of the bioactive agent, or otherwise enhance its pharmacological properties (e.g., increase in vivo half-life, reduce toxicity, etc.).

30 Another aspect of the present invention includes formulations that provide for the sustained release of DSB repair antagonists. Examples of such sustained release formulations include composites of biocompatible polymers, such as poly(lactic acid), poly(lactic-co-glycolic acid), methylcellulose, hyaluronic acid, collagen, and the like. The structure, selection and use of degradable polymers in drug delivery vehicles have been reviewed in several publications, including, A. Domb et al., Polymers for Advanced Technologies 3:279-292 (1992). Additional guidance in selecting and using polymers in pharmaceutical formulations can be found in the text by M. Chasin and R. Langer (eds.), "Biodegradable Polymers as Drug Delivery Systems," Vol. 45 of "Drugs and the Pharmaceutical Sciences," M. Dekker, New York, 1990. Liposomes may also be used to provide for the sustained release of DSB repair antagonists. Details concerning how to use and make liposomal formulations of drugs of interest can be found in, among other places, U.S. Pat. Nos. 4,944,948; 5,008,050; 4,921,706; 4,927,637; 4,452,747; 4,016,100; 4,311,712; 4,370,349; 4,372,949; 4,529,561; 5,009,956; 4,725,442; 4,737,323; 4,920,016. Sustained release formulations are of particular interest when it is desirable to provide a high local concentration of DSB repair antagonist, e.g., near a tumor, site of inflammation, etc.

31 Where diagnostic, therapeutic or medicinal use of the presently described agents, or derivatives thereof, is contemplated, the bioactive agents may be introduced in vivo by any of a number of established methods. For instance, the agent may be administered by inhalation; by subcutaneous (sub-q); intravenous (I.V.), intraperitoneal (I.P.), or intramuscular (I.M.) injection; or as a topically applied agent (transdermal patch, ointments, creams, salves, eye drops, and the like).

32 Additionally, an alternative means for employing the presently disclosed anti-proliferation agents includes the use of vectors to directly insert genes encoding the agents into target cells (e.g., gene therapy). For example, when the tumor cells express the genes encoding the desired sequences, DSB repair will be disrupted and the tumor cell will die. Alternatively, one could attack tumor cells using a strategy conceptually similar to that disclosed in U.S. Pat. No. 5,529,774 herein incorporated by reference. In brief, cells that produce transducing virus encoding sequence that disrupts DSB repair may be implanted at or near the tumor mass. As the producer cells continue to elaborate virus, the growing tumor cells are infected and effectively killed as they expressing the agent that blocks DSB repair. The above methodology has proven useful in the treatment of glioblastomas and other tumors of the brain by using retroviral vectors to selectively target actively replicating tumor cells. A similar methodology could be used to deliver antisense sequences that target (and thus inhibit) the expression of Rad51 or any of the proteins involved in the Rad51 or Rad52 pathways.

33 The mammalian Rad51- or Rad52-mediated repair pathways, and the associated proteins, are essential for cell proliferation or viability. These DNA repair pathways most likely function by repairing DSB via homologous recombination between sister chromatids during S/G.sub.2 (recombinational repair); however, during G.sub.1, the repair of DSB may also occur via nonhomologous recombination (nonhomologous end joining). The nonhomologous recombination pathway was once thought to be the major repair pathway in mammalian cells.

Much of this belief stems from gene targeting data that demonstrated homologous recombination to be less frequent than random or illegitimate recombination (Bradley et al., 1992, Bio/Technology 10:534-39). Other data demonstrated that chromosomal DSB frequently were joined without homology or with only very short stretches of homology (Rouet and Jasin, 1994, Mol. Cell. Biol. 14:8096-8105). DNA-dependent protein kinase (DNA-PK) is critical for nonhomologous but not homologous repair of DSB (Liang et al., 1996, Proc. Natl. Acad. Sci. USA 93:8929-33). A biphasic response to ionizing radiation was observed in DNA-PK-deficient cell lines with resistance in late S phase suggesting that DNA-PK functions in G.sub.1 and another repair pathway functions in S phase (Jeggo, 1990, Mutation Research 239:1-16). DNA-PK is composed of a catalytic subunit called DNA-PK.sub.cs and a DNA end-binding subunit called Ku which is a heterodimer of Ku70 and Ku86 (Park et al., 1996, J. Biol. Chem. 1996:18996-19000, for review, see Roth et al., 1995; Shen et al., 1996. Analysis of DNA-PK activity has come from said (severe combined immunodeficient) mice which are deficient in DNA-PK.sub.cs (Kirchgessner et al., 1995, Science 267:1178-82), and Ku86-deficient mice (Nussenzweig et al., 1996, Nature 382:551-55; Zhu et al., 1996, Cell 86:379-89). Both said and Ku86-deficient mice are immune deficient due to a defect in repair of DSB generated during V(D)J recombination. Unfortunately, it is impossible to analyze V(D)J recombination in rad51-mutant mice or cells; however, it is unlikely that MmRad51 plays a role in this process since MmRad51 localizes to the nucleus in late G.sub.1 through G.sub.2 (Yamamoto et al., 1996, 251:1-12), and V(D)J recombination occurs in G.sub.0 /G.sub.1 (Schlissel et al., 1993, Genes & Dev. 7:2520-32). In general, said and Ku86-deficient cells do have similarities to MmRad51-deficient cells. All are hypersensitive to ionizing radiation, and Ku86-deficient cells were prematurely senescent in tissue culture, indicating a similar function. However, since scid and Ku86-deficient mice and cells were viable and MmRad51-deficient cells were not, the consequences of removing the putative homologous recombination pathway to repair DSB appears to be more vital than the removal of the nonhomologous pathway.

34 The presently described DSB repair antagonists are particularly deemed useful for the treatment of cancer. Cancers that may be treated by the methods of the invention include, but are not limited to: Cardiac: sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma; Lung: bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma; Gastrointestinal: esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma); Genitourinary tract: kidney (adenocarcinoma, Wilm's tumor [nephroblastoma], lymphoma, leukemia), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma); Liver: hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastom, angiosarcoma, hepatocellular adenoma, hemangioma; Bone: osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor, chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors; Nervous system: skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma [pinealoma], glioblastoma multiforme, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), spinal cord (neurofibroma, meningioma, glioma, sarcoma); Gynecological: uterus (endometrial carcinoma), cervix (cervical carcinoma,

pre-tumor cervical dysplasia)! ovaries (ovarian carcinoma [serous cystadenocarcinoma, mucinous cystadenocarcinoma, endometrioid tumors, celioblastoma, clear cell carcinoma, unclassified carcinoma], granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma [embryonal rhabdomyosarcoma], fallopian tubes (carcinoma); Hematologic: blood (myeloid leukemia [acute and chronic], acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic syndrome), Hodgkin's disease, non-Hodgkin's lymphoma [malignant lymphoma]; Skin: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles, dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis; and Adrenal glands: neuroblastoma.

35 In addition to cancer, the presently disclosed compounds are effective against any of a wide variety of hyperproliferative disorders including, but not limited to: autoimmune disease, arthritis, inflammatory bowel disease, proliferation induced after medical procedures, including, but not limited to, surgery, angioplasty, and the like.

36 The anti-cancer application of agents that functionally disrupt mammalian Rad51, Rad52 or any member in the DSB repair pathway, requires that DSB repair remains equally critical in cancer cells. Cancer cells lack many of the normal cell cycle regulatory mechanisms that are critical to controlling proliferation, and inducing programmed cell death, and it remains possible that the absence of these mechanisms renders Rad51 and/or Rad52 function nonessential. The protein p53 is central to regulation of the cell cycle, and stimulation of cell death in response to DNA damage including DNA damaged by ionizing radiation (reviewed by Ko and Prives, 1996, Genes & Develop. 10:1054-72). p53 is the most commonly mutated gene in cancer cells (Donehower et al., 1992, Nature 356:215-21; Vogelstein, 1990, Nature 348:681-682) and mutations in p53 are known to increase cell proliferation and promote chromosomal instability (Harvey et al., 1993, Oncogene 8:2457-67).

37 The early lethal phenotype in rad51.sup.M1 mutant embryos and cells may be stimulated by a cell cycle response to unrepaired DNA damage. DNA damage was shown to inhibit progression through the cell cycle, demonstrating a relationship between DNA lesions and cell cycle proteins (Carr and Hoekstra, 1995, Trends in Cell Biology 5:32-40). In mitotically dividing budding yeast cells, a single DSB in a dispensable plasmid was sufficient to induce cell death, partly under the control of Rad9 (Bennett et al., 1993, Proc. Natl. Acad. Sci. USA 90:5613-17; Schiestl et al., 1989, Mol. Cell. Biol. 9:1882-9654, Weinert and Hartwell, 1988, Science 241:317-22). In mammalian cells, the tumor suppressor gene, p53, responded to DNA damage induced by T-radiation by delaying the cell cycle, or inducing programmed cell death (Kastan et al., 1991, Cancer Research 51:6304-11; Kuerbitz et al., 1992, Proc. Natl. Acad. Sci. USA 89:7491-95). These responses may be critical the tumor suppressor function of p53 (Baker et al., 1990, Science 249:912-15; Lowe et al., 1994, Science 266:807-10, Symonds et al., 1994, Cell 78:703-11). Induction of p53 after exposure to ionizing radiation and restriction endonuclease suggest that the formation of DSB may initiate a p53 response (Lu and Lane, 1993, Cell 75:765-78).

38 p53 was at least partly responsible for regulating the rad51.sup.M1 phenotype because development was extended from the early egg cylinder stage to the head fold stage in a p53-mutant background. However, the double-mutant embryos died from either accumulation of DNA damage resulting in metabolic incompetence and mitotic failure, or p53-independent regulation. Murine embryonic fibroblasts, generated from double-mutant embryos, failed to proliferate and were completely senescent in tissue culture; thus, demonstrating that MmRad51 function was critical in cells that exhibit chromosomal instability and accelerated proliferation. It is therefore likely that disruption of MmRad51 or any other protein in its pathway or disruption of any protein-protein interaction important in the DSB repair pathway results in reduced proliferation or decreased cell viability. This feature remains true even in cells with reduced

capacity to regulate the cell cycle.

39 The present invention is further illustrated by the following examples, which are not intended to be limiting in any way whatsoever.

40 5.0. EXAMPLES

41 5.1. Cloninc of the Mouse MmRAD51 cDNA

42 The MmRAD51 cDNA sequence was cloned and used to generate an expression vector. The 5' end of cDNA was amplified by RT-PCR from mouse testis RNA and was then used as a probe to screen a mouse brain cDNA library. One clone was identified and sequenced. The coding sequence was identical to MmRAD51 disclosed in published reports (Morita et al., 1993; Shinohara et al., 1993); however, the clone contained about 300 additional base pairs of 5' noncoding sequence and about 400 extra base pairs of 3' noncoding sequence (FIG. 1).

43 5.2. The Use of a Yeast Two-Hybrid Screen to Isolate Proteins That Associate with MmRad51

44 ScRad51 was shown to self-associate as well as associate with other proteins such as ScRad52 and ScRad55 (Donovan et al., 1994; Hays et al., 1995; Johnson and Symington, 1995; Milne and Weaver, 1993; Shinohara et al., 1992). Kluyveromyeces lactis RAD51 and RAD52 did not rescue a rad51.DELTA. rad52.DELTA. strain of S. cerevisiae and over expression of ScRAD51 suppressed rad55 and rad57 mutant yeast which indicates interacting proteins are necessary (Donovan et al., 1994; Hays et al., 1995). Also, Dmc1 and ScRad51 colocalized to the synaptonemal complex which suggested that they act together during meiotic recombination (Bishop, 1994).

45 The modified yeast two-hybrid system was used to isolate proteins that associate with mammalian Rad51 which is a genetic screen for determining protein-protein interactions (Harper et al., 1993). One of the proteins is a hybrid of the GAL4 DNA-binding domain fused to MmRad51 (the "bait"). The other is a hybrid of the GAL4 transactivating domain fused to an embryonic or a T cell cDNA library (the "prey"). The bait and prey were co-expressed in HF7c yeast that contained two reporters, HIS3 and lacZ fused to the GAL4 promoter and grown in media lacking histidine and containing 25 mM 3-AT (an antimetabolite; 3-amino-1,2,4-triazole). Functional GAL4 was created when the DNA binding domain and the transactivation domain were juxtaposed, ideally by a MmRad51-protein interaction. Such an interaction induced the HIS3 and lacZ genes allowing a positive colony to survive in medium lacking histidine and to turn blue in X-Gal (5-bromo-4-chloro-3-indolyl-.beta.-D-galactosidase).

46 Seven specific clones were isolated from this screen. A 13.5 day embryonic cDNA library (500 .mu.g) was transfected into 5.times.10.sup.6 cells and plated onto forty 15 cm plates. A T cell cDNA library (400 .mu.g) was transfected into 4.times.10.sup.6 cells and plated onto twenty 15 cm plates. A total of 80 His.sup.+ colonies grew in about 3 days. Of these, 40 turned blue after about 5 to 30 minutes of exposure to X-gal. These colonies were tested for specificity by transfecting HF7c cells without bait or with a nonspecific bait (E12). Nonspecific associations were observed in 20 clones. The inserts in the other clones were sequenced and 13 were out of frame and seven were in frame. The sequences for the remaining seven clones were screened in the GCG data base. Homologues were found for four clones and three clones were novel (Table 1). The protein produced by clone 1 was 100% homologous to MmRad51 which showed that the screen was successful because RecA and ScRad51 are both known to self-associate. The protein produced from clone 2 was 100% homologous to a metal response element binding protein, M96 (Inouye et al., 1994, DNA and Cell Biol. 13(7):731-742). The function of M96 is unknown. The protein produced from clone 3 was 48% homologous to human XP-G (ERCC-5) and 45% homologous to chicken Histone H1. A mutation in XP-G is responsible for the genetic disorder xeroderma pigmentosum (Cleaver, 1994; Cleaver and Kraemer, 1995, In The metabolic basis of inherited disease, p. 4393-4419, 7th ed. McGraw-Hill, New York.). XP-G is a homologue of the S. cerevisiae excision repair protein,

ScRad2 which is a ssDNA endonuclease. It is possible that MmRad51 repairs single-strand breaks as well as double-strand breaks and that single-strand breaks can initiate recombination. Histone H1 is a component of the nucleosome and comprises a group of related proteins that vary in tissues and are poorly conserved across species. The length of DNA may be affected by Histone H1 binding to the linker region and joining adjacent nucleosomes. The protein produced from clone 4 was 100% homologous to the human breast cancer gene, BRCA2 (Tavtigian et al., 1996, Nat. Gen. 12:333 -337; Wooster et al., 1995, Nature 378:789-792). The function of Brca2 is unknown; however, like p53, it is a tumor suppressor gene and may therefore regulate the cell cycle in response to DNA damage. Thus, the observed association with a DNA repair gene, MmRad51, is consistent with such an activity.

TABLE 1

Clones isolated from the yeast two-hybrid screen

Clone	Homology	Library
1	100% to MmRad51	T cell
2	100% to M96 embryo	
3	45% to Histone H1, 48% to to XP-G	embryo
4	100% to Brca2	T cell
5	novel	T cell
6	novel	embryo
7	novel	embryo

47 Clones isolated from a yeast two-hybrid screen with MmRad51 as the "bait" and an embryonic or T cell cDNA library as the "prey". The inserts obtained from the prey were sequenced and compared to sequences in the GCG data base. The measured extent protein homology is listed. All clones strongly associated with MmRad51 in the N-terminal region (amino acids 1-43). Colonies grew with in three days in 3-AT, and cells generally stained blue after about 5 minutes of X-gal exposure.

48 5.3. Deletion Analysis of MmRad51 to Isolate the Protein Association Region

49 A deletion analysis was performed to isolate the MmRad51 self-association domain. Full length MmRAD51 was used as the bait and deletions of MmRAD51 were the prey (FIG. 2). The "prey" MmRad51 deletions were individually co-transfected with the bait into HF7c cells. The relative levels of .beta.-galactosidase activity were measured for the MmRad51 deletion proteins as compared to full length MmRad51 which was considered to have 100% activity. Expression of the C-terminal region, TR43-339 and TR131-339 did not result in blue yeast cells after 10 hours, and the relative .beta.-galactosidase activity was about 1%, or the same as for the nonspecific bait, E12. However, expression of the N-terminal region, TR1-43, stained yeast cells blue in less than 5 minutes and the relative .beta.-galactosidase activity was 43%. Interestingly, a sequence containing more of the N-terminal region of the protein, TR1-93, caused the yeast cells to stain blue after about 30 minutes of X-gal exposure, and reduced the relative .beta.-galactosidase activity to about 4%. In similar experiments, TR1-131 and TR1-175 respectively displayed 11% and 9% of the .beta.-galactosidase activity of the positive control. Nevertheless, these data indicated that the N-terminal region was responsible for MmRad51 self-association. It also appeared that amino acids 43-93 inhibited self-association and that this inhibition was relieved by adding more of the C-terminal region of the protein. These data indicated that MmRad51 was functionally conserved with ScRad51 since the self-association domain was also in the N-terminal region for both proteins even though these regions did not display conserved amino acid sequences.

50 The other six proteins listed in Table 1 were tested to determine if they

interacted with the N-terminal region of MmRad51. All six strongly interacted with TR1-43; thus, the most N-terminal 43 amino acids were responsible for all the MmRad51 protein-protein interactions observed. Given the high level of homology shared between the human and murine Rad51 proteins (in the important N-terminal self-association region, the proteins only differ at amino acid positions 10 and 46 where the human sequence respectively contains an asparagine in lieu of the serine, and a phenylalanine in place of the tyrosine encoded by the mouse protein--both relatively conservative replacements), the presently described results should reflect the results expected from similar studies using the human Rad51 protein.

51 6.4. Transfection of Mouse Embryonic Stem Cells with Altered Alleles of Mammalian rad51

52 Both MmRad51 and ScRad51 self-associate using their respective N-terminal regions. This observation supports the hypothesis for that these proteins remain functionally conserved. Functional conservation was further tested in the RecA core homology domain. In ScRad51, the RecA core homology region was shown to be essential for the repair of DSB. The gene rad51K-A191 was altered in the first ATP-binding motif, and a conserved Lysine was changed to an Alanine. The expression of rad51K-A191 in wild-type yeast cells dominantly impaired the repair of DNA damage and generated a rad51 null phenotype. Nonproductive protein-protein interactions were probably responsible for the dominant negative phenotype because rad51K-A191 was shown to associate with wild-type ScRad51 and ScRad52. If the MmRad51 structural domains were similar to ScRad51, then disruption of the conserved Lysine in the first ATP-binding motif should result in a null phenotype because of the nonfunctional associations with wild-type MmRad51 or other proteins in this pathway such as mouse Rad52 or Brca2. A null rad51 mutation resulted in a severe cell proliferation defect that prevented propagation of mutant mouse cells in tissue culture. Therefore, cells that expressed a dominant negative rad51 allele should not be recovered due to this proliferation defect.

53 Altered alleles of mammalian rad51 that were engineered to be dominate negative were expressed in mouse embryonic stem cells. Due to the severity of the null phenotype, these experiments were designed to measure the absence of transfected cells by statistically relevant numbers. The first experiment measured the transfection efficiencies of vectors that expressed altered mammalian rad51 as compared to a vector that expressed wild-type mammalian RAD51, or vector alone. The altered transgenes, rad51TR1-131 and rad51K-A134, contained a functional protein binding region and a nonfunctional RecA homology region. For rad51TR1-131, a C-terminal truncation was made in the first ATP-binding domain (FIG. 1). For rad51K-A134, the conserved Lysine in the first ATP-binding motif was changed to an Alanine (for review, see Donovan and Weaver, 1994). rad51K-A134 more strongly associated with full length MmRad51 than rad51TR1-131 as measured using the yeast two-hybrid system with about 90% relative .beta.-galactosidase activity (FIG. 1). The altered and wild-type transgenes were cloned into a CMV expression vector with a neomycin phosphotransferase (neo) cassette (pcDNA3 from invitrogen). Transfected embryonic stem (ES) cells were selected in G418 and colonies were counted 9 days later. The altered transgenes generated 20-30% fewer G418.sup.r colonies as compared to colonies resulting after transfection with wild-type MmRAD51 or vector alone in three experiments. Variations of 20-30% in transfection frequencies are commonly observed and are consequently not determinative in and of themselves. However, this minimal reduction could also indicate that the toxic product of the altered transgenes was produced in sufficient quantities to stop cell proliferation. However, if the transgene product was truly toxic, then why did 70-80% of the cells survive in selection media? The transgene may be silent while neo gene is expressed. The transgene may be disrupted upon integration into the chromosome or by chromosomal positional effects. In addition, strong expression of the transgene may be required to observe a phenotype while only weak expression of neo may be required for positive selection. Another experiment was needed to circumvent these possible problems.

54 6.5. Targeting the Expression Vectors to the HPRT Locus

55 Another experiment was developed to compare the targeting frequencies of vectors that expressed altered mammalian rad51 with vectors that expressed wild-type mammalian RAD51 or MC1tk (Herpes Simplex Virus type 1 thymidine kinase). The transgenes were targeted to the hypoxanthine phosphoribosyltransferase locus, HPRT (Melton et al., 1984, Proc. Natl. Acad. Sci. USA 81:2147-2151). Targeting the transgenes to HPRT would decrease the likelihood of disruption upon integration and Southern analysis could also be used to verify the integrity of the integration event (FIG. 3). The transgenes would also be located to a favorable environment for expression since HPRT is a house keeping gene, and thus all of the transgenes would be affected to the same degree by chromatin positional effects. The transgenes were cloned into the bacterial plasmid of an insertion vector that targeted HPRT (IVH). There were 6.9 kb of HPRT sequences that contained a neo cassette in exon 3. Therefore, upon linearization using a unique site in the homology region (an engineered NotI site), both insertion and replacement events could be recovered.

56 The targeting vectors were linearized in the HPRT homology region and transfected into ES cells. Transfected cells were selected for by growth in medium containing G418, and targeted cells were selected in medium containing G418+6-thioguanine (TG). G418 resistant (G418.sup.r) colonies were counted to measure the transfection efficiency and TG.sup.r +G418.sup.r colonies were counted to measure the targeting frequency.

TABLE 2

Targeting frequencies

	No. of Exp.	total Exps.	total G418.sup.r	frequency TG.sup.r + G418.sup.r	target relative to IVH-tk
IVH-tk A	2	4088	338	1/12	NA
IVH-51TA	1	636	34	1/19	-37%
IVH-51KA	2	2792	106	1/26	-54%
IVH-tk B	2	1200	124	1/10	NA
IVH-51TA	2	472	22	1/21	-52%
IVH-51KA	2	1504	62	1/24	-58%
IVH-tk C	2	6016	264	1/23	NA
IVH-51WT	2	4840	192	1/26	-12%
IVH-51TA	2	2584	70	1/37	-38%
IVH-51KA	2	3664	48	1/76	-70%
IVH-tk D	2	6744	414	1/16	NA
IVH-51KA	2	4848	136	1/37	-57%
IVH-tk E	2	2624	186	1/14	NA
IVH-51WT	2	1456	84	1/17	-18%
IVH-51TA	2	2208	96	1/23	-39%
IVH-51KA	2	1376	52	1/26	-46%
IVH-tk F	2	1664	156	1/11	NA
IVH-51WT	2	752	60	1/12	-8%
IVH-51TA	2	760	34	1/22	-50%
IVH-51KA	2	544	30	1/18	-39%

57 Electroporation: 10 .mu.g of NotI cut DNA/10.sup.7 cells/ml PBS, 575 V/cm and 500 .mu.F. Each experiment (exps. A-F) shows results from electroporations that were done on the same day with a common batch of ES cells under identical conditions to eliminate variability. NA, not applicable.

58 The targeting frequencies of vectors that contained altered rad51 alleles were compared to control vectors (Table 2). Vectors that contained altered rad51

alleles were IVH-51TR1-131 (contains rad51TR1-131) and IVH-51KA (contains rad51K-A134). Control vectors were IVH-51WT (contains wild-type MmRAD51), and IVH-tk (contains MC1tk). The relative targeting frequencies (TG.sup.r +G418.sup.r /G418.sup.r colonies) were determined using IVH-tk efficiency as 100%. The relative targeting frequencies were reduced by 13+/-3.6% for IVH-51WT (average of three experiments), 43+/-6.4% for IVH-51TR1-131 (average of 5 experiments) and 54+/-7.6% for IVH-51KA (average of six experiments).

59 Southern analysis was performed on TG.sup.r +G418.sup.r clones to verify targeting and to identify the different targeting patterns (FIG. 3). Several types of recombination patterns were possible. A vector insertion event would integrate the entire vector to form a duplication of HPRT homology (Hasty et al., 1992, Molec. and Cell. Biol. 12:2464-2474). The vector may integrate on the 5' long arm or the 3' short arm (rarely observed). These integration patterns were combined since both integrate the transgene in between the duplication. A gene replacement event would introduce the neo but not the transgene and thus, provided a control. Modified events, that were not predicted by either pattern could also occur, and an intact transgene may or may not be introduced.

60 Comparison of the targeting patterns for the four vectors indicated that the transgene product was toxic for both rad51TR1-131 and rad51K-A134. The relative percentage of clones targeted with IVH-51TR1-131 and IVH-51KA that contained the transgene (vector insertion) decreased, and the relative percentage of targeted clones that did not contain the transgene (gene replacement) increased relative to controls. For both IVH-tk and IVH-51WT, targeting usually occurred by vector insertion (75% and 80%, respectively), rarely by gene replacement (14% and 17%, respectively), or more rarely by a modified event (6% and 8%, respectively). However, for IVH-51TR1-131 and IVH-51KA the relative frequency of targeted events that occurred by vector insertion decreased (68% and 45%, respectively), and gene replacement events increased (27% and 41%, respectively). The relative frequency of modified events also increased for clones targeted with IVH-51KA (14%). Therefore, the altered transgenes rarely integrated into the target locus as compared to the controls.

61 5.6. A High Percentage of Transfected Clones did not Express the Transpene

62 A statistically significant reduction in targeting frequency was observed using vectors that contained the altered rad51 alleles as compared to the wild-type allele or MC1tk. In addition, altered transgenes were introduced into HPRT for a lower percentage of the targeted clones as compared to the controls. However, targeted clones were generated that appeared to incorporate the altered transgenes intact. There are several possibilities for survival: 1) A small mutation may have been generated in the transgene; 2) The chromatin structure of the transgene may have been altered during the targeting event to silence the transgene (or vice-versa); 3) Position effect variegation may inhibit transcription of the transgene, but not neo.

63 Expression of MC1tk was tested in clones targeted with IVH-tk to determine the fraction of clones that do not express the transgene. Sixty-two TG.sup.r +G418.sup.r clones were grown in replica plates, one without FIAU and one with FIAU, to distinguish clones that lost or maintained HSV-1 thymidine kinase activity. A large percentage of clones (42%) survived in FIAU demonstrating that the IVH-51TR1-131 and IVH-51KA targeting frequencies were reduced to background levels. Therefore, all of the cells targeted with either IVH-51TR1-131 and IVH-51KA that express the transgene were probably not recovered.

64 6.7. Application of Molecules that Disrupt Mammalian Rad51 and/or Rad52 Function for Cancer Therapeutics

65 The rad51.sup.M1 mutation reduces proliferation and promotes cellular senescence, even in a p53 mutant background. In addition, rad51 dominant negative alleles also display this phenotype by presumably forming nonproductive protein associations with Rad51 and other proteins like Rad52,

M96 and Brca2. Therefore, it is likely that the disruption of mammalian Rad51, mammalian Rad52 (or any protein in the DSB repair pathway mediated by these proteins) will reduce cell proliferation or induce cell death, and thus be suitable as a cancer therapeutic. In addition, the disruption of any protein-protein association important for mammalian Rad51 function or mammalian Rad52 function will also reduce cell proliferation or induce cell death, and thus be suitable as a cancer therapeutic.

66 Additionally, dominate negative alleles of rad51 may be used to express cancer therapeutics that reduce cell proliferation or induce cell death. An expression vector that codes for a dominate negative rad51 allele may be introduced into cancer cells, or an mRNA that codes for a dominate negative rad51 allele may be introduced into cancer cells, or a dominate negative Rad51 protein may be introduced into cancer cells. Several examples of such dominate negative rad51 alleles are presently disclosed. Of these alleles, the protein encoded by rad51K-A131 appears to have the strongest self-association, and proved toxic to proliferating cells. In fact, any rad51 allele that rendered the RecA homology region nonfunctional but preserved the N-terminal protein association region should reduce cell proliferation or induce cell death and could thus be used as a cancer therapeutic.

67 In addition to subtle alterations in the RecA core homology region of mammalian Rad51, C-terminal truncations in mammalian rad51 may also be used to reduce cell proliferation and/or induce cell death. rad51TR1-131 demonstrated a toxic effect on cells even though it had a relatively weak interaction with MmRad51 which suggested that the phenotype might be caused by nonfunctional self-associations, or nonfunctional associations with other proteins such as Rad52, M96 and Brca2. rad51TR1-43 had a strong interaction with MmRad51 and may be more effective as a cancer therapeutic than rad51TR1-131. In fact, any C-terminal truncation that preserves the protein interacting region of Rad51 may be used as a dominate negative allele for cancer therapy. Additionally, fusion of the N terminal domain of mammalian Rad51 to the 16 or 60 amino acids of the 3rd helix of the antennapedia protein may promote entry into the nucleus (Derossi et al., 1994, J. Bio. Chem. 269:10444-10450).

68 Mammalian Rad51 interacts with other proteins besides itself, and disruption of these interactions could be used to reduce cell proliferation or induce cell death. Other proteins interacting with mammalian Rad51 include but are not limited to mammalian Rad52, Brca2 and M96.

69 The identification of other interacting proteins will further elucidate the pathway and present greater opportunities to disrupt this pathway for the purpose of hindering cell proliferation. Since mammalian Rad52 associates with mammalian Rad51 and other proteins (Park et al., 1996; Shen et al., 1996), dominant alleles of mammalian Rad52 may also hinder cell proliferation or induce cell death. Such alleles could also be used for cancer therapeutics. In fact, dominant alleles of any protein that associates with mammalian Rad51, Rad52 or any other protein in these pathways, may be expected to hinder cell proliferation or induce cell death. Thus, all of the above molecules collectively define a new class of therapeutic agents for the treatment of proliferative disorders, viral infection (especially HIV infection), and cancer.

70 EQUIVALENTS

71 The foregoing specification is considered to be sufficient to enable one skilled in the art to broadly practice the invention. Indeed, various modifications of the above-described makes for carrying out the invention which are obvious to those skilled in the field of microbiology, biochemistry, organic chemistry, medicine or related fields are intended to be within the scope of the following claims. All patents, patents applications, and publications cited are herein incorporated by reference.

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```

CLAIMS:

What is claimed is:

1. A method of screening for compounds that disrupt mammalian double stranded break repair, comprising:

a) contacting a mammalian cell with a compound to be screened;

b) assaying for microsatellite formation in said cell;

c) assaying for chromosome loss in said cell;

d) assaying said compound for the disruption of strand exchange in an in vitro assay; and

e) correlating assay results from steps b, c and d to identify a compound that disrupts mammalian double stranded break repair.

2. A method according to claim 1 wherein said compound is a molecule produced by cells.

3. A method according to claim 1 wherein said compound was chemically synthesized.

4. A method of screening for compounds that disrupt the function of mammalian Rad51, comprising:

a) in a yeast two-hybrid system wherein mammalian Rad51 is one component, identifying a protein that interacts with mammalian Rad51;

b) contacting said system with a compound to be screened; and

c) correlating assay results from step b to identify a compound that disrupts the function of mammalian Rad51.

5. A method according to claim 4 wherein said compound is a molecule produced by cells.

6. A method according to claim 4 wherein said compound was chemically synthesized.

7. A method of screening for compounds that disrupt the function of mammalian Rad52, comprising:

a) in a yeast two-hybrid system wherein mammalian Rad51 is one component identifying a protein that interacts with mammalian Rad51;

b) contacting said system with a compound to be screened; and

c) correlating assay results from step b to identify a compound that disrupts the function of mammalian Rad51;

whereby disruption of the function of mammalian Rad51 is correlated with the disruption of mammalian Rad52.

8. A method according to claim 7 wherein said compound is a molecule produced by cells.

9. A method according to claim 7 wherein said compound was chemically synthesized.

10. A method of screening for compounds that disrupt the function of mammalian Rad51, comprising:

a) in a biochemical binding assay system wherein Rad51 is one component, identifying a compound that is associated with mammalian Rad51;

b) contacting said system with a compound to be screened; and

c) correlating assay results from step b to identify a compound that disrupts the function of mammalian Rad51.

11. A method according to claim 10 wherein said compound is a molecule produced by cells.

12. A method according to claim 10 wherein said compound was chemically synthesized.

13. A method of screening for compounds that disrupt the function of mammalian Rad52, comprising:

a) in a biochemical binding assay system wherein Rad51 is one component, identifying a compound that is associated with mammalian Rad51;

b) contacting said system with a compound to be screened; and

c) correlating assay results from step b to identify a compound that disrupts the function of mammalian Rad51;

whereby disruption of the function of mammalian Rad51 is correlated with the disruption of mammalian Rad52.

14. A method according to claim 13 wherein said compound is a molecule produced by cells.

15. A method according to claim 13 wherein said compound was chemically synthesized.